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*STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC SUBSTANCES. VII. PRODUCTION OF ACTINOMYCIN BY DIFFERENT ACTINOMYCETES**, †

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The ability of organisms belonging to different species or even different genera to produce the same antibiotic has been definitely established for the fungi. This is true of penicillin production by different species of *Penicillium* and *Aspergillus;* of clavacin, produced by a variety of species belonging to these two as well as other genera; of citrinin; and of other antibiotics. In the case of spore-forming bacteria, which received considerable attention during the last few years, the problem is more difficult because of the greater complexity of the substances produced. It is difficult to say as yet whether some of the products described in the literature, such as subtilin and bacitracin, are chemical individuals or mixtures. Likewise it is difficult to say whether such mixtures may contain one or more of the recognizably pure substances such as gramicidin, tyrocidine, or gramicidin S along with other active and inactive polypeptides; tyrothricin, for example, has been shown to contain gramicidin and tyrocidine, along with other less well-defined substances.

The identity of an antibiotic produced by different species of actinomycetes is complicated by the difficulty of recognizing distinct species. It is believed, however, that the data presented in this paper tend to suggest that different species, or at least what may be considered as such, may produce the same type of antibiotic. The differences in yield and especially in the impurities accompanying the particular antibiotic tend to emphasize further the differences in species, or at least in strain specificity.

In 1940, the isolation from the soil of an actinomyces that had strong antibiotic properties was reported.[1] This substance was designated as actinomycin and the organism described as *Actinomyces* (*Streptomyces*) *antibioticus*.[2] Since then some 10,000 cultures of actinomycetes have been isolated from soils, composts, and other substrates, and tested for anti-

biotic properties, and in only two other instances was the production of this antibiotic definitely established.

The production of actinomycin can be detected by its antibiotic spectrum, by its characteristic pigmentation, and by its chemical properties, since it can be isolated from the medium and crystallized.[3] Its antibacterial properties comprise a very high activity against gram-positive bacteria and rather low activity against gram-negative organisms.

In a search for substances possessing activity against viruses,[4] a culture of actinomyces was obtained and found to produce the typical red substance described originally as actinomycin A. The new culture (S-4) resembled *S. antibioticus* in some of its morphological and cultural properties, although it was not identical with it. It produced on synthetic media, for example, a deep black zone in the aerial mycelium. The exact significance of this zone has not yet been determined.

TABLE 1

BACTERIOSTATIC ACTION OF THREE ACTINOMYCIN PREPARATIONS

	BACTERIOSTATIC ACTION BY AGAR DILUTION METHOD, DILUTION UNITS PER GRAM		
CRYSTALLINE ANTIBIOTIC	ACTINOMYCIN FROM CULTURE S-4	ACTINOMYCIN A FROM *S. antibioticus*	ACTINOMYCIN FROM 36-G
E. coli	<10,000	<5,000	<30,000
A. aerogenes	<10,000	<10,000	<30,000
S. aureus	>20,000,000	>20,000,000	>3,000,000
B. subtilis	>20,000,000	>20,000,000	>3,000,000
B. mycoides	>20,000,000	>20,000,000	>3,000,000
S. lutea	>100,000,000	>100,000,000	.....
	Bacteriostatic action by cup method, diameter of zone in mm.		
0.1 mg./ml.	31.4	31.6	
0.01 mg./ml.	27.2	27.5	

By following the original procedure[3] for isolating actinomycin A, a crystalline red solid was obtained which had a melting point of 252°C. and which gave no depression in the melting point when mixed with an authentic specimen of actinomycin A. The antibiotic spectra of the two substances and the quantitative concentration of pure actinomycin, as measured by the cup method against *B. subtilis*, were found to be identical, as shown in table 1.

In the course of isolation of the actinomycin from S-4, some material soluble in petroleum ether was also obtained. This fraction was previously designated actinomycin B; it was a mobile yellow oil and was produced by either strain. The bacteriostatic spectra of the two fractions (after repurification) were also similar, both giving 20,000 to 60,000 units per gram, against gram-positive bacteria. Since activity of this magnitude could result from an admixture of inert material with as little as 0.5 per cent of

actinomycin A, it is probable that the activity of this fraction is due entirely to some contamination with actinomycin A. Since even this limited activity gave exactly the same type of spectrum as actinomycin A, the second fraction may, therefore, be considered as an impurity of the latter. Because of this and in order to avoid future confusion, it is proposed to abandon the term "actinomycin B" and to change the name "actinomycin A" to "actinomycin."

The amounts of actinomycin produced by the newly isolated S-4 strain as well as by the original *S. antibioticus* 3435 strain, grown both in shaken and in stationary cultures, were then investigated. Starch tryptone medium was used, with 0.25 per cent agar for stationary cultures. The results, summarized in table 2, show that strain S-4 produces nearly 10 times as much actinomycin as the original *S. antibioticus*. The substance produced by S-4 can also be isolated more readily. The actinomycin produced by the two strains differed also in another respect: the substance formed by

TABLE 2

PRODUCTION OF ACTINOMYCIN BY 3 DIFFERENT CULTURES OF STREPTOMYCES

STRAIN S-4		*S. antibioticus* 3435		STRAIN 36-G
SHAKEN	STATIONARY	SHAKEN	STATIONARY	STATIONARY
Activity of culture filtrate, dilution units (*B. subtilis*)				
3,000	3,000	200	300	300
Yield of isolated crude actinomycin, milligrams per liter				
200	170	114	100	66
Total activity of crude actinomycin produced, dilution units				
1,200,000	1,000,000	140,000	120,000	250,000

S-4 was readily crystallized from acetone-ether mixtures after the removal of the B-fraction, whereas that produced by *S. antibioticus* could not be crystallized until after chromatographic separation because of the presence of tarry impurities.

In the course of this work, three methods of extraction of the actinomycin were used: (1) extraction of cultures with ether in stationary flasks; (2) continuous extraction with ether; (3) extraction with ethyl acetate. Methods 2 and 3 proved to be about equally satisfactory and were superior to method 1 on the basis of completeness of removal of the substances from the culture filtrate. The nature of the product seemed to be independent of the method of extraction.

More recently, another culture was isolated which produced actinomycin when grown on a glucose-tryptone medium, both in stationary soft agar cultures and in a submerged state. This culture, 36-G, was isolated from soil. It was markedly different from both *S. antibioticus* and S-4. It was non-chromogenic and did not form the typical sporulating aerial mycelium

characteristic of *S. antibioticus*. The straight conidiophores were arranged irregularly on the aerial mycelium. The yield of actinomycin (66 mg. per liter) given by this culture was less than that of the other 2 cultures. The activity of the culture filtrate (300 *B. subtilis* units per ml.) was similar to that of *S. antibioticus*. The antibiotic spectrum of the culture filtrate and of the crystalline product was that typical of actinomycin, as shown in the tables.

Summary.—Actinomycin is produced by different species of the genus *Streptomyces*. The yield and purity of the antibiotic depend upon the nature of the culture. One organism yielded about 10 times as much actinomycin as the original *S. antibioticus*. Another culture gave a lower yield than *S. antibioticus*, but a purer product was obtained. The nature and activity of the second fraction accompanying the actinomycin, namely actinomycin B, also varied for the different cultures; however, its antibiotic spectrum was similar to that of actinomycin. Because of the insignificant yields of the B fraction, and because of the suggestion that its activity is due to traces of actinomycin A present as impurities, it is proposed to abandon the name of "actinomycin B" and to change the name of "actinomycin A" to "actinomycin."

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GROWTH REQUIREMENTS OF VIRUS-RESISTANT MUTANTS OF ESCHERICHIA COLI STRAIN "B"

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The isolation of a number of virus-resistant mutants from a virus-sensitive strain of *Escherichia coli* (strain *B*) has been described in a previous publication.[1] The mutant strains were obtained by using, as selective

agents, three bacterial virus strains (bacteriophages), all of which are active on the parent organism. Lysis of the sensitive cells allowed the resistant mutants to develop as secondary colonies. The mutant strains differed from the original strain by their resistance to one, any two or all three viruses, in all possible combinations. All mutants were found to possess the morphological characteristics of the parent strain. Various qualitative fermentation tests carried out on representative strains also failed to demonstrate essential differences. The mutants were tested for their ability to grow in a synthetic minimal medium containing inorganic salts, asparagine and dextrose buffered at pH 7.0. This medium will be referred to as the asparagine medium.[2] These tests revealed that many of the mutants were unable to develop, although the medium supported heavy growth of the parent strain. The addition of as little as 0.005 per cent Difco yeast extract to the minimal medium enabled all mutants to show good growth. In an attempt to determine what indispensable substances the mutants had apparently lost the ability to synthesize, representative strains were systematically tested in a synthetic minimal medium to which vitamins, amino acids, hydrolyzed yeast nucleic acid and hydrolyzed casein were added singly and in various combinations. The synthetic medium used in these tests contained inorganic salts, dextrose and ammonium chloride in place of asparagine, and will be referred to as the ammonium medium.[3] All substances tested were found to be inactive under the conditions of the tests. The present paper deals with a continuation of the investigation of the growth factor requirements of the virus-resistant mutants of strain *B*.

Resistance Patterns of Deficient Mutants.—The 27 mutant strains used in the present study failed to grow in either synthetic medium without the addition of nutrient broth or minute amounts of yeast extract. All deficient mutants were resistant to virus *T*1[4] and 22 strains were also resistant to one or more of the other viruses for which the *B* strain may serve as host. On the basis of resistance tests carried out with high titre stocks of seven viruses active on *B*,[5] the 27 mutants represent four different patterns of resistance as follows: 5 strains resistant to virus *T*1; 2 to *T*1, *T*3 and *T*4; 3 to *T*1 and *T*6; and 17 resistant to *T*1, *T*3, *T*4 and *T*7. These patterns of resistance are consistent with the resistance groups discussed by Demerec and Fano.[4]

Seventeen of the mutants were derivatives of *B*/1, strain no. 1. These were isolated as double mutants by subjecting the *B*/1 strain to the action of either virus *T*2 or *T*7 which, in addition to *T*1, were used as selective agents in the isolation of the mutants. Many of the derivatives of *B*/1 may be the result of independent duplication of mutations. *T*6 was not used in the isolation of this group of mutants. The occurrence of 3 strains resistant to *T*1 and *T*6 may be explained by the observation of Delbrück[6]

that resistance to $T6$ is facultatively coupled with resistance to $T2$. These three strains were originally isolated as $B/2/1$. It has been our experience that mutants resistant to $T2$ have a high rate of back mutation to sensitivity. In these cases it may be assumed that the organisms isolated were actually $B/2$, $6/1$ and that the loss of resistance to $T2$ did not affect the resistance to $T6$ or $T1$. It has been reported[4] that mutants of B which are resistant to virus $T1$ fall into two distinct types, those which acquire resistance to $T1$ only, and those which simultaneously acquire resistance to $T5$. All the strains unable to grow in synthetic media were found to be sensitive to $T5$.

Tryptophane as Growth Factor and the Influence of Nitrogen Source.—All substances tested in the previously reported studies[1] gave completely negative results with the exception of *dl*-tryptophane which, in a concentration of 1 microgram per milliliter, gave inconclusive growth with the mutant strains used in the tests. On testing the 27 deficient strains for their ability to grow in the ammonium medium containing 30 micrograms *l*(−)-tryptophane per milliliter it was found that, with one exception, all strains showed some development after 14 hours' incubation at 37°C. and good growth at the end of 38 hours. Other amino acids[7] added to the ammonium medium[6] in concentrations of 100 micrograms per milliliter failed to allow development of any of the mutant strains tested.

In an attempt to determine the concentration of *l*(−)-tryptophane necessary for development of $B/1$, strain no. 1, a graded series of tryptophane concentrations ranging from 200 to 0.01 microgram per milliliter was set up in 1-ml. amounts in the ammonium medium and in the asparagine medium. After 20 hours' incubation at 37°C. visible growth was observed in the ammonium medium in all tubes containing 20 micrograms of tryptophane while the series in the asparagine medium showed growth at 0.2 microgram per milliliter. Incubation over a period of 140 hours permitted visible growth in the ammonium series to 1.56 micrograms tryptophane per milliliter. Development in this medium, however, was greatly reduced in all tubes containing less than 12.5 micrograms of tryptophane per milliliter and in the asparagine medium at concentrations of less than 1.13 micrograms of tryptophane per milliliter.

Comparative studies on the growth of the parent strain in the two synthetic media had shown NH_4Cl and asparagine to be entirely comparable as a nitrogen source. The observation that the asparagine medium permitted growth of the deficient strain at a much lower concentration of tryptophane than did the ammonium medium indicated that the deficient strains, in addition to requiring tryptophane as a growth factor, may have nitrogen requirements which differ from those of the parent strain. Therefore, a more extensive investigation of the nitrogen requirements of the deficient and the parent strains was undertaken.

Amino Acids as Nitrogen Source in Combination with NH_4Cl.—Preliminary studies were carried out with *B*/1, strain no. 1, in which 21 amino acids were individually tested for their ability to serve as an additional nitrogen source when added to the ammonium medium supplemented with tryptophane. Each amino acid was tested at a concentration of 100 micrograms per milliliter in two series of media, one of which contained 20 micrograms *l*(−)-tryptophane per milliliter and the other 100 micrograms per milliliter. The tests were carried out in 10-ml. volumes in large test tubes with aeration. Growth was measured after 24 hours' incubation at 37°C. by centrifuging each culture in its entirety in Hopkins' vaccine tubes. Nearly all amino acids tested gave some increase in the cell yield of the organism over that obtainable in the controls containing tryptophane and NH_4Cl as sole nitrogen source. An exception was *dl*-norleucine which inhibited growth at the concentration tested. Ten amino acids, however, permitted appreciably increased growth of *B*/1. These were *l*(−)-histidine, *l*(+)-arginine, *l*(−)-leucine, *dl*-phenylalanine, *dl*-valine, glycine, *dl*-serine, *l*(+)-glutamic acid, *l*(−)-proline and *l*-asparagine.

Selected Amino Acids as Sole Nitrogen Source for the Parent Strain.—These ten amino acids as well as *l*(−)-tryptophane were tested as sole nitrogen source for the parent strain. They were added in 1 mg. per milliliter amounts to 10-ml. volumes of the minimal medium with the NH_4Cl omitted. Cell volume measurements of *B*, determined as above, indicated that the eleven amino acids tested could be arbitrarily divided into three groups on the basis of their ability to serve as nitrogen sources for strain *B*. Group I, consisting of asparagine, arginine, serine and glycine gave good growth. Proline and glutamic acid in group II gave fair growth. Very little or no growth was obtained with histidine, tryptophane, leucine, valine and phenylalanine which comprised group III. The inability of the parent strain to develop on group III amino acids cannot be considered as due to the necessity of accessory growth factors since it is fully able to grow in inorganic nitrogen and glucose. Parallel tests in which the amino acids were added to the minimal medium containing NH_4Cl showed that all eleven amino acids permitted good growth in the presence of the ammonium salt. Therefore, the failure of the group III amino acids to permit growth when present as sole nitrogen source may be explained on the basis that their nitrogen is unavailable rather than that these amino acids are toxic to the organism. The data obtained in one representative experiment are presented in table 1. It will be noted that although tryptophane is essential for the growth of *B*/1, it is not a suitable source of nitrogen for *B* in the absence of other nitrogen sources.

Selected Amino Acids as Sole Nitrogen Source for B/1.—Essentially the same grouping was obtained when the ten amino acids were tested individually for their ability to serve as sole nitrogen sources for *B*/1 in the

minimal medium containing no NH_4Cl. The tests with this organism were carried out in the same manner as those for the parent strain except that 100 micrograms tryptophane per milliliter were added as a growth factor. Although cell yields of $B/1$ are low with NH_4Cl as sole source of nitrogen, it is apparent that NH_4-nitrogen may be used in the presence of certain amino acids. As is seen in table 1, NH_4Cl appears to be essential for the utilization of histidine, leucine, valine and phenylalanine by $B/1$. It is not clear whether NH_4-nitrogen is used in the presence of the other amino acids since growth is heavy in its absence. It was observed, however, that in all cases visible growth was obtained earlier in tubes containing NH_4Cl than in parallel tubes in which this salt was omitted.

TABLE 1

GROWTH OF *B*, *B*/1, STRAIN NO. 1 AND *B*/1/7, 3, 4, STRAIN NO. 1A, ON SELECTED AMINO ACIDS IN A MINIMAL MEDIUM WITH AND WITHOUT NH_4Cl

SUBSTANCE ADDED, 1 MG. PER ML.	*B*		*B*/1, NO. 1		*B*/1/7, 3, 4, NO. 1A	
	$-NH_4Cl$	$+NH_4Cl$	$-NH_4Cl$	$+NH_4Cl$	$-NH_4Cl$	$+NH_4Cl$
l(+)-Arginine	41.5	46.0	26.0	33.0	20.0	27.0
l-Asparagine	29.0	28.0	17.0	12.0	15.0	30.0
dl-Serine	36.0	36.0	26.5	30.0	26.5	26.5
Glycine	26.5	30.0	12.0	17.0	15.0	20.5
l(−)-Proline	5.0	26.0	8.0	12.0	9.0	20.0
l(+)-Glutamic acid	3.5	29.5	11.0	16.5	7.5	18.0
l(−)-Histidine	0.0	19.0	0.5	30.5	3.5	23.0
l(−)-Tryptophane	0.5	26.5				
l(−)-Leucine	0.5	22.5	1.0	11.0	7.0	15.0
dl-Valine	1.0	32.0	1.0	19.5	6.0	24.0
dl-Phenylalanine	0.5	22.5	0.5	15.0	6.0	24.0
Control	0.0	24.0	0.5	3.5	3.5	9.0

Growth in $mm.^3$ cells per 10 ml. in 24 hours.

Media used in testing *B*/1 contained 100 micrograms *l*-tryptophane per milliliter.

Media used in testing *B*/1/7, 3, 4 contained 100 micrograms *l*-tryptophane and 100 micrograms *l*-proline per milliliter.

Tryptophane Requirements with Optimal Nitrogen Source.—It is apparent from these results that the amount of growth of the tryptophane requiring strains is limited not only by the amount of tryptophane present in the medium, but also by the nature of the substances serving as source of nitrogen. Therefore, in testing the efficiency of *l*(−)-tryptophane as a growth factor for the deficient strains the tests were carried out in the ammonium medium to which 1 mg. of hydrochloric acid–hydrolyzed yeast extract per milliliter was added. This preparation was entirely free of tryptophane but together with NH_4Cl served as a completely adequate source of nitrogen.

Figure 1 presents the growth response of *B*/1, strain no. 1, to varying concentrations of *l*(−)-tryptophane in this medium. The determinations

were carried out in 1-ml. volumes in small test tubes without aeration and cell counts were made by the usual plating method at the end of 24 hours' incubation at 37°C. From the data presented it is seen that, provided a suitable nitrogen source is available in sufficient quantity, the growth of this mutant is proportional to the amount of tryptophane added and that with 0.25 microgram of *l*(−)-tryptophane 10^8 cells developed in 24 hours.

Irreplaceability of Tryptophane.—In the case of certain microörganisms which require tryptophane for growth it has been shown that this amino acid can be replaced by substances postulated as intermediate in the synthesis of tryptophane. Indole is utilized by some strains of *B. typhosum*,

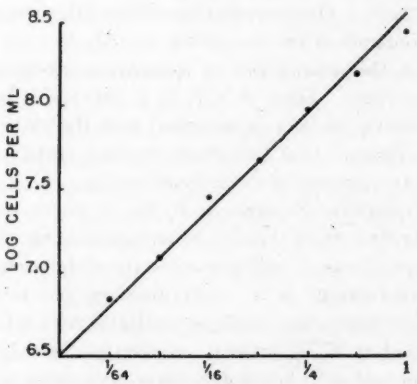

FIGURE 1

Growth of *B*/1, no. 1 in varying concentrations of *l*(−)-tyrptophane.

C. diphtheriae and staphylococcus,[8] and either indole or anthranilic acid can replace tryptophane for certain species of lactic acid bacteria.[9] Two tryptophaneless mutant strains of the ascomycete *Neurospora crassa* have been described,[10] one of which is able to use indole and the other either indole or anthranilic acid in place of tryptophane. Tatum and Bonner[11] have presented evidence that in this mold the biosynthesis of tryptophane takes place by a direct reaction between indole and *l*(−)-serine.

Eight tryptophaneless mutants of *B* were tested for their ability to grow in the ammonium medium, supplemented by 1 mg. per milliliter hydrochloric acid-hydrolyzed yeast extract, to which was added *l*(−)-tryptophane, indole,[12] or anthranilic acid[12] in 50 micrograms per milliliter amounts. Additional tests were carried out using *B*/1, strain no. 1, in the ammonium medium to which was added alpha methyl indole,[12] xanthurenic acid,[12]

tryptamine hydrochloride,[12] indole acetic acid,[12] indole propionic acid,[12] indole pyruvic acid,[12] indole butyric acid[12] or indole + *dl*-serine. In no case was growth of any of the tryptophane-deficient mutants of *B* obtained in the absence of tryptophane.

With one exception tryptophane has been found to satisfy the growth factor requirements of all mutants unable to grow in either minimal medium. In addition, tryptophane is required in much higher concentrations when NH_4Cl is supplied as the nitrogen source than the concentration necessary to support good development in the presence of certain amino acids.

All strains are capable of development in the ammonium medium supplemented with small amounts of tryptophane and hydrochloric acid-hydrolyzed yeast extract. This combination of nutrilites was found to satisfy the growth requirements of the one strain, *B*/1/7, 3, 4, no. 1A, which was unable to grow in the ammonium or asparagine medium supplemented with tryptophane alone. Since *B*/1/7, 3, 4, no. 1A, is incapable of development in synthetic media supplemented with the hydrolyzed yeast extract alone, it is apparent that this strain requires tryptophane as well as some factor or factors present in yeast hydrolyzate.

Proline and Tryptophane Required by B/1/7, 3, 4, No. 1A.—In order to determine whether this strain requires other amino acids as growth factors in addition to tryptophane, 1-milliliter amounts of the ammonium medium containing 100 micrograms of *l*(−)-tryptophane per milliliter and 100 micrograms of individual amino acids per milliliter were set up in small test tubes and incubated at 37°C. without aeration. The only tubes showing development at the end of 24 hours were those containing tryptophane plus *l*(−)-proline and tryptophane plus *l*(−)-hydroxyproline. Incubation for 144 hours resulted in a slight increase of those two tubes but no visible growth with any of the other 19 amino acids tested. Much higher cell concentrations were obtained with proline than with hydroxyproline. The optimum concentrations of tryptophane and proline required for the growth of this strain have not been determined.

Selected Amino Acids as Sole Nitrogen Source for B/1/7, 3, 4, No. 1A.—The tryptophane-proline-deficient mutant was also tested for its ability to utilize the group of ten amino acids as sole nitrogen sources. Tests were carried out as for *B* and *B*/1, but with tryptophane and proline, 100 micrograms of each per milliliter, added as growth factors to the minimal medium. From the data presented in table 1 it is seen that the amino acids fall into essentially the same grouping as with the other two strains. Growth in the control is, however, greater than that in the controls of *B/1*. This may be due to the presence of proline in an amount above the growth factor requirements for this amino acid, the excess being utilized as nitrogen source. This is reflected in the growth of this strain with most of the group III amino acids in the media without NH_4Cl. The addition

of NH_4Cl to the media permitted good development of *B*/1/7, 3, 4, strain no. 1A, with all amino acids tested.

Discussion.—Certain mutations from virus sensitivity to virus resistance in the *B* strain of *E. coli* may result in a loss of the ability of the mutant to synthesize factors essential for growth. This is indicated by the observation that many of the mutant strains are unable to develop in the absence of tryptophane. In the present study all tryptophaneless mutants have been found to be resistant to *T*1 but sensitive to *T*5. Mutants resistant to *T*1 and *T*5 are capable of growth with inorganic nitrogen and glucose and therefore may be presumed to have retained the synthetic capacity of the parent strain. It would therefore appear that resistance to *T*1 may be the result of different mutations—one type blocking the synthesis of an essential metabolic product in addition to preventing the synthesis of specific constituents essential to the reaction of the cell and a specific virus. Further studies, however, will be required to determine whether or not the loss of the capacity of synthesizing tryptophane is directly correlated with a specific resistance pattern. The deficient strain requiring proline in addition to tryptophane was one of 17 *B*/1/7, 3, 4 strains. Tryptophane alone satisfied the need for accessory growth factors for the other 16 strains, indicating that there are different mutations involving resistance to *T*7 and that the need for proline may be associated with one specific type of these mutations.

That other factors in the synthetic capacity of the tryptophaneless mutants may also become limiting as a result of the mutation is indicated by the observation that even though the growth factor requirements be satisfied, the deficient mutants, in contrast to the parent strain, are incapable of growing to high cell concentrations when NH_4Cl is supplied as sole source of nitrogen. Supplementation with amino nitrogen results in greatly increased growth. Therefore it would appear that the amination capacity of these strains has become altered. In this respect it is interesting that the presence of NH_4-nitrogen appears to be essential for the utilization of certain of the amino acids.

Summary.—Certain virus-resistant mutants derived from a virus-sensitive strain of *E. coli* were found to require accessory growth factors although the parent strain is capable of full development in a medium containing inorganic nitrogen and dextrose. The 27 strains used in this study have apparently lost the ability to synthesize tryptophane. One strain was found to require proline in addition to tryptophane. All of the tryptophaneless strains are resistant to virus *T*1 and sensitive to *T*5.

That other factors in the synthetic capacity of the mutants differ from those of the parent strain is evidenced by the observation that organic as well as ammonia nitrogen is essential for full development of the mutant strains. In a medium containing adequate nitrogen sources the addition

of 0.25 microgram of *l*(−)-tryptophane gives a development of 10^8 cells in 24 hours.

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[*] Fellow in the Medical Sciences of the National Research Council.

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[2] The asparagine medium consisted of: *l*-asparagine 0.2 per cent, dextrose 0.4 per cent, Na_2HPO_4 (anhydrous) 0.6 per cent, KH_2PO_4 0.3 per cent, $MgSO_4$ 0.005 per cent, NaCl 0.005 per cent, glass distilled water.

[3] The ammonium medium consisted of: NH_4Cl 0.1 per cent, dextrose 0.4 per cent $NaHPO_4$ (anhydrous) 0.6 per cent, KH_2PO_4 0.3 per cent, $MgSO_4$ 0.02 per cent, NaCl 0.05 per cent, glass distilled water.

[4] Demerec, M., and Fano, U., *Genetics*, **30**, 119–136 (1945).

[5] Delbrück, M., *Biological Reviews*, **21**, 30–40 (1946).

[6] Delbrück, M., unpublished data.

[7] The following amino acids were tested: *dl*-alanine, *l*(+)-arginine, *l*-asparagine, *l*(+)-aspartic acid, *l*(−)-cystine, *l*(+)-glutamic acid, glycine, *l*(−)-histidine, *l*(−)-hydroxyproline, *dl*-isoleucine, *l*(−)-leucine, *dl*-leucine, *l*(+)-lysine, *dl*-methionine, *dl*-norleucine, *dl*-phenylalanine, *l*(−)-proline, *dl*-serine, *dl*-threonine, *l*(−)-tryptophane, *l*(−)-tyrosine, *dl*-valine.

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[12] Kindly provided by Dr. E. L. Tatum, Osborn Botanical Lab., Yale Univ.

EXPERIMENTS ON SEXUAL ISOLATION IN DROSOPHILA. VII. THE NATURE OF THE ISOLATING MECHANISMS BETWEEN DROSOPHILA PSEUDOOBSCURA AND DROSOPHILA PERSIMILIS

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An ever increasing number of cases is being described in the current literature of pairs of exceedingly similar species that coexist at the same locality. The morphological similarity sometimes reaches the point of virtual identity, in other cases very minor differences exist in regard to the characters of eggs, larvae or adults. Adherents of a strictly morphological species concept interpret such findings as intraspecific differentiation and apply the term "physiological races" to the members of such pairs of extremely similar species. However, it has been found in all well-studied

cases that partial or complete reproductive isolation exists between the members of these pairs of populations and that no hybrids are found in nature in the regions of distributional overlap, even in the cases where hybrids can be obtained experimentally. The adherent of a biological species concept is forced to regard as species sympatric populations that are reproductively completely isolated. The two species *Drosophila pseudoobscura* and *D. persimilis* are such a pair of species. Although the morphological differences between these species[1] are very slight, still not a single hybrid has yet been found in the wide area of overlap in western America. In the laboratory the two species can be crossed fairly easily, and the hybrid females are fertile and can be backcrossed. Hybrid F_1 males, however, are completely sterile.[2]

Sex Behavior in Intraspecific Crosses.—A study of the normal sex behavior of the two species would seem a necessary prerequisite of a study of their mutual incompatibilities and isolating mechanisms. Unfortunately, however, the sex physiology of the two species is by no means exhaustively known.

Some data are presented in the following sections on the sexual physiology and the isolating mechanisms of an orange-eyed strain of *D. pseudoobscura* Frolova descended from flies collected at Piñon Flats, San Jacinto Mountains, California, and a wild strain of *D. persimilis* Dobzhansky and Epling from Stony Creek, north of the Sequoia National Park, California. Only these two strains of the two species were studied. Courtship in *D. melanogaster* and many other species has been described by Sturtevant[3, 4, 5] and in *D. virilis* by Stalker.[6] For *D. pseudoobscura* and relatives data were presented by several authors.[2, 7, 8]

Age at Sexual Maturity.—Flies of the species *D. pseudoobscura* become sexually mature earlier than flies of the closely related species *persimilis* and *miranda*. Dobzhansky and Koller[7] found that in a strain of *D. pseudoobscura* kept at 24.5°C. about 36% of the females were fertilized after $1^1/_2$ days, 62% after 2 days, 82% after 3 days. In a strain of *D. miranda* only 4% of the females were fertilized after 2 days, 56% after 3 days, 66% after 4 days. *D. persimilis* seems to be still slower. Even 3-day-old flies are rarely fertilized and flies at least 5 days old (preferably 6 or 7) have to be used, to be sure that they are sexually mature. This poses the awkward problem that flies of different chronological age must be used to be sure that they are approximately of the same physiological age. Therefore the females of *D. pseudoobscura* used for most of the experiments were 5 or 6 days old and those of *persimilis* 7 days old.

Sexual Activity.—Both males and females of the orange-eyed strain of *D. pseudoobscura* were sexually much more active than flies of the *D. persimilis* strain. It did not prove feasible to measure this difference quantitatively, but if the following arbitrary point values are given to the sex drives:

♂ *pseudoobscura* 120, ♀ *pseudoobscura* 80, ♂ *persimilis* 50, ♀ *persimilis* 10, and a value of −110 to the isolating mechanisms in the interspecific matings, we obtain the following combined values:

persimilis ♂ with *persimilis* ♀ : 50 + 10 = 60
persimilis ♂ with *pseudoobscura* ♀ : 50 + 80 − 110 = 20
pseudoobscura ♂ with *pseudoobscura* ♀ : 120 + 80 = 200
pseudoobscura ♂ with *persimilis* ♀ : 120 + 10 − 110 = 20

The ratios 60:20 (=3:1) and 200:20 (=10:1) are fairly close to the observed ones in double choice experiments.[8] They are not entirely exact, however, since the actual values of the isolating mechanisms are fairly different in reciprocal crosses (above listed always as −110). The incompatibility between *pseudoobscura* ♂ and *persimilis* ♀ is much greater than that between *persimilis* ♂ and *pseudoobscura* ♀.

Sexual activity in *Drosophila*, unfortunately, happens to be a somewhat unpredictable factor; particularly in well-aged flies. It was high on certain days, low on others even though all experimental conditions including temperature were seemingly identical. The time of day seemed to play some rôle: sexual activity is apparently higher in the morning and evening than during the middle of the day. Quantitative experiments, to be strictly comparable, should be conducted not only at the same temperatures but also at the same hour of the day.

Mating Behavior of Males.—It has been shown by Sturtevant[3, 4, 5] that the following elements are the most frequent components of the mating behavior of *Drosophila* males. In various combinations they are found in most species of the large genus *Drosophila*.

"*Vibrating.*"—The male faces the female (usually from the side), extends one wing at about right angles to his body, and vibrates it for a few seconds. The wing is then returned to the normal position. The vibrated wing is usually the one nearer the head of the female. Both wings are rarely vibrated simultaneously and only in a few species.

"*Waving.*"—The wing is also extended laterally, but is not vibrated.

"*Scissors Movements.*"—Both wings are rapidly opened and closed like a pair of scissors.

"*Licking.*"—The male licks with his proboscis the ovipositor of the female.

"*Circling.*"—A rapid sideways movement of the male from a frontal or lateral position to one behind the female (usually followed immediately by copulation).

Of these five elements only *vibrating* and *circling* were found in *D. pseudoobscura* and *persimilis*. It was not possible to discover any difference between the two species, either in the qualitative or quantitative aspects of the courtship.

Copulation in the two species proceeds as described by Sturtevant[3] for *D. melanogaster*. The male, standing behind the female, bends up his abdomen underneath, until its tip faces forward. The phallus is then thrust into the female ovipositor, and after its intromission the male parts the wings of the female and mounts. In some other species the female opens her wings spontaneously and in still others the male mounts the back of the female before intromission of the phallus.

Termination of a normal copula is apparently always initiated by the male by extracting his phallus. Usually he succeeds in doing this in 20 or 30 seconds, but it may require 3 or 4 minutes in exceptional cases. Males of the observed strain of *D. persimilis* were usually rather inactive after completed copulation and spent much of their time in preening. No second copulation was recorded during the observation periods. Males of the observed strain of *D. pseudoobscura* sometimes engaged in a second copulation within 30 or 40 seconds after completion of the first, and in a third copulation after completing the second. If no receptive females are available, males may become completely quiescent within about 20 minutes after a period of great excitement and much displaying.

Female Behavior.—Receptive females stand still, turn the tip of the abdomen toward the male, lift it and partly extrude the ovipositor ("invitation display"). With ready males this will result in almost instantaneous copulation, other males—particularly young males and males of other species—may pay no attention to the female's overtures. Many males copulate without a preceding invitation display by the female. Copulating females ward off other males by stretching the middle pair of legs sideways. Non-receptivity is indicated by the following actions of females: walking away rapidly, wing-flicking, depressing the tip of the abdomen toward the ground, or a combination of these methods. Females of *D. pseudoobscura* and *D. persimilis*, which had just completed copulation, were non-receptive at least for one hour. They were receptive when tested again 24 hours later. Sturtevant[4] found that in *D. repleta* and *affinis* the same pair may copulate twice within 10 minutes. Repeated copulations were also found in other species.

Length of Copulation.—Sturtevant[4,5] reports that the length of copulation of various species of *Drosophila* may vary between 1 minute (*lutzii, hydei*) and 55 minutes (*immigrans*).

First copulations of *D. pseudoobscura* males lasted 4′30″, 4′35″, 4′55″, 5′55″, 6′10″, 6′15″, 6′15″, 7′0″, 7′10″, 8′0″, 9′15″, 10′5″, 11′15″ in homogamic matings (median 6′15″). No copulations with *D. persimilis* ♀ were timed. Second and third successive copulations usually last shorter than the first. The duration of successive copulations was: 4′30″, 3′26″, 2′20″; −4′55″, 3′10″, 4′15″, −7′10″, 4′40″; −8′0″, 5′20″. Temperatures were about 21–24°C., but unfortunately were not accurately recorded.

First copulations of *D. persimilis* males lasted 4′40″, 5′0″, 5′35″, 5′40″, 6′10″, 6′10″, 6′10″, 6′25″, 6′35″, 6′40″, 7′0″, 7′20″, 7′20″, 8′20″, 8′30″, 9′25″ in homogamic matings (median 6′30″). One copulation with a *pseudoobscura* female lasted 7′35″. There is thus no striking difference between the two species. Stalker[6] likewise found no significant difference in length of copulation between the closely related species *D. virilis* and *D. americana*.

Species Recognition and Psychological Isolating Mechanisms.—Most modern authors assume that "psychological barriers" ("species recognition") prevent or reduce the frequency of matings between members of closely related species of insects. These terms signify a crude concept of the interplay between male and female, which has no reality. The term "recognition" implies consciousness and the ability of making judgments, for which no evidence exists in *Drosophila*. Rather it must be assumed that the male stimulates the female by specific pre-copulatory displays and that the female reacts by specific responses indicating a state of receptiveness. This interpretation assumes that successful copulation is the result of a chain of interactions between specific stimuli produced by the male and adequate responses of the female which in turn stimulate the male.

If the reproductive isolation between *D. pseudoobscura* and *D. persimilis* is partly or entirely due to psychological isolating mechanisms, an analysis of the pre-copulatory display of males and females should reveal differences. The above-described observations indicate that there are no visible differences in the courtship behavior of the two species. This is, in a way, not surprising since the major elements of the courtship, vibrating, circling, scissors movement, and licking in various combinations are widespread in the genus *Drosophila*.

The possibility remains that auditory, olfactory or other factors provide the stimulation necessary to limit copulation to encounters between conspecific individuals. To test this possibility a series of multiple choice experiments were undertaken.[8]

Multiple Choice Experiments.—Males of one species were given the opportunity to mate under varying conditions with females of two species. When an equal number of females of *D. pseudoobscura* and *D. persimilis* was placed in a vial with food together with males of *D. pseudoobscura*, it was found[8] that about 11 times as many *pseudoobscura* females were inseminated as *persimilis* females. Males of *persimilis* fertilized about 3 times as many of their own as *pseudoobscura* females. These control experiments, as well as those of earlier authors,[2, 7] permit thre econclusions. First, that sexual isolation between the two species is not nearly as complete under experimental conditions (only one kind of male present) as in nature. Second, that mating between the flies is not random, but indicative of highly developed discrimination. Third, that conspecific matings are much more

frequent than heterogamic matings. The experiments, however, do not elucidate the reason for the higher frequency of conspecific pairings.

Questions that need to be answered are the following: Is a fly stimulated by an individual of the opposite sex regardless of the species to which it belongs? If there is a difference between species in stimulation, how large is it and to what extent is it responsible for the reproductive isolation of the species concerned? Are male and female equally involved in the difference which seems to exist between conspecific and non-specific pre-copulatory stimuli? Which sense organs are important as receptory mechanisms for these stimuli?

Methods.—Different techniques were employed in the attempt to elucidate these questions. In mass experiments 10 females of each of two species were placed in a vial of food with several males of one of these species. The females were dissected after an interval sufficient to permit fertilization of about 50 per cent of them, and the percentage of fertilized females in the lots of the two species determined. This indirect method was supplemented by direct observation. A special observation chamber was constructed which consisted of a wax ring between two parallel glass plates. The size of the ring was adjusted not to exceed the field of vision of a low-power binocular microscope. The flies were introduced into the wax ring through a funnel-like opening which could be closed by a stopper. In this chamber flies remained in good physical condition for hours, but most observation periods were terminated after 30 minutes and the flies replaced by new ones. This observation chamber permitted the observation at a 7-fold magnification of every detail of the movements of 4–8 flies, all of them at all times completely in focus.

A different observation technique was employed where numerical counts were more important than a study of the details of behavior. Batteries of ten glass vials without food were used, each one of them containing the same combination of flies. The ten vials were observed simultaneously and the number and sequence of events recorded. All transfers of aged flies were made without etherization. All tested flies were virgin at the beginning of the observation periods.

The Rôle of the Sense Organs.—Vision: The two species *D. pseudoobscura* and *D. persimilis* are indistinguishable to the human eye. There is no significant difference in the insemination ratio of mixed cultures kept in the light and such that were kept in the dark.[8] This indicates that vision is not essential for species discrimination. However, absolute proof for the rôle of light can be obtained only if the dark-light experiment could be repeated after the complete elimination of all other sense organs, because it is conceivable that other senses might take over the function of vision after its elimination.

Hearing: When a male of *D. pseudoobscura* or *D. persimilis* courts a

female he spreads a wing and vibrates it. Since Reed, *et al.*,[9] have shown that the means of the wing areas are different in the two species, the possibility exists that the pitch of wing vibration is also different and may serve as a "species recognition signal." However, it was shown[8] in experiments involving wingless males, that actually a smaller percentage of alien females was inseminated and that the total number of inseminated females had dropped. It seems on the basis of these and other observations that it is the rôle of the vibrating wings to stimulate the females and to get them into a receptive state. Furthermore, the overlap in the normal wing pitch variability of the two species is much too large for a character to be useful in species discrimination.

Smell: It is well known that specific scents play an important rôle in the courtship of many insects. It was therefore tried to test what effect on species discrimination the elimination of the olfactory sense would have. The olfactory organ of *Drosophila* is located in the terminal segment of the antennae,[10, 11] and can be removed rather easily. Four males of *D. pseudoobscura*, each placed with ten females of *D. pseudoobscura* and *D. persimilis* one day after the complete amputation of all segments of both antennae, performed as shown in table 1.

TABLE 1

RECORDS OF *D. pseudoobscura* AND *D. persimilis* FEMALES INSEMINATED BY *D. pseudoobscura* MALES WITHOUT ANTENNAE

HOMOGAMIC FEMALES		HETEROGAMIC FEMALES		ISOLATION INDEX
N	%	*N*	%	
38	52.7	39	10.2	0.68

N = number of females; % = percentage of inseminated females.

Although the isolation index is significantly lower than in control experiments[8] (where it is 0.80 or higher), still five times as many conspecific as alien females were inseminated by males without antennae. If these findings could be confirmed with more extensive material, they would indicate the following facts: lack of the olfactory sense in males increases the number of heterogamic crosses, which implies that to a certain extent the olfactory sense of males is involved in species discrimination. However, species discrimination is still high even without the olfactory apparatus.

Absence of Species Discrimination in Courting Males.—The reported experiments indicate that none of the investigated sense organs and display mechanisms had a controlling influence on species discrimination. It appeared therefore advisable to record quantitative data on species discrimination by direct observation of courting males, both in the observation chamber and in the vial batteries previously described. The observations gave no indication of species discrimination by males, as documented by the following excerpts from my protocols. (The term "incomplete copula-

tions" is applied to copulations which are typical in every respect and include intromission of the male phallus and mounting, but are terminated after 1–2 seconds.)

"July 19 (10:15 A.M.). 2 ♂ *pseudoobscura* (8 days old) placed with 4 ♀ *persimilis* (6 days old). Males very active and aggressive. No less than 50 incomplete copulations observed during a 30-minute period. No sperm found in genital tract of females. Females seem to coöperate fully during the incomplete copulations. They go through the same motions as when being courted by their own males, such as stopping, lifting the abdomen and turning it slightly toward the courting male."

Males display to alien females and attempt to copulate with them even when females of their own species are available. This is particularly true when males of *D. persimilis* are placed with the very active *pseudoobscura* females together with their own rather quiet and sluggish females:

"July 20 (10:26 A.M.). 3 ♂ *persimilis* (8 days old), 3 ♀ *persimilis* (8 days old), 2 ♀ *pseudoobscura* (7 days old). 30-minute period. Male *persimilis* rather active, display both to *persimilis* and *pseudoobscura* females. After 15 minutes first and only copulation (homogamic). Males display during the last fifteen minutes almost entirely to *pseudoobscura* females."

"July 21 (8:06 A.M.). One hour. 3 ♂ *persimilis* (8 days old), 3 ♀ *persimilis* (8 days old), 3 ♀ *pseudoobscura* (8 days old). There are numerous incomplete copulations of *persimilis* males with *pseudoobscura* females. One such heterogamic copulation is successful. During the whole hour there is not a single persistent attempt of a *persimilis* ♂ to copulate with a *persimilis* ♀."

"August 2 (7:42 P.M.). One hour. Ten vials each with 1 *persimilis* ♂ (7 days old), 1 *persimilis* ♀ (7 days old), 1 *pseudoobscura* ♀ (6 days old). During first 10 minutes males display almost exclusively to *pseudoobscura* females. In three vials there are very frequent incomplete copulations with *pseudoobscura* ♀. The males in two vials are entirely inactive, in six of the other vials they clearly concentrate their attention on *pseudoobscura* females. However, not a single successful heterogamic copulation occurred. The only attempted homogamic copulation was at once successful. The *persimilis* ♀ of one vial walked repeatedly past the *persimilis* ♂, who persistently displayed to *pseudoobscura* ♀ and payed no attention to his own female."

All female flies were dissected 13 hours after end of observation. Five *persimilis* ♀ and one *pseudoobscura* ♀ were found to be inseminated.

These records show clearly that males display without apparent discrimination to females of both species, and that in fact the majority of the display of *persimilis* ♂ are directed toward *pseudoobscura* ♀, which are more active than their own. However, the overwhelming majority of the

heterogamic copulations in which these displays culminate remain incomplete.

Functional Difficulties.—Entomologists have long contended that the peculiarities of the sexual armatures might and occasionally do prevent interspecific crosses. The notion of a complete fit of a lock and key mechanism of male and female genitalia in its most exaggerated form is undoubtedly not correct, as pointed out by Dobzhansky[12] and other authors. However, mechanical difficulties do exist in most interspecific matings and reduce their efficiency as indicated by Sturtevant[4] and described in detail by Stalker[6] for cross matings between *D. virilis* and *D. americana*. Observations of the cross matings of *D. pseudoobscura* and *D. persimilis* fully confirm this. The following protocol may be added to those recorded above.

"July 24 (9:54 A.M.). 30 minutes. 3 *pseudoobscura* ♂ (6 days old), 4 *persimilis* ♀ (7 days old). During the first 7 minutes only one male is active. At least 16 incompiete copulations are counted during this period. In the next 10 minutes all 3 males are active, attempting to copulate with 2 of the females. I count 48 genital contacts during this 10-minute period and undoubtedly overlooked several others during this frenzy of activity. None of these 70 or more contacts leads to a completed copulation. All flies are quiescent during the final 6 minutes of the observation period. The females do not run away from the males, in fact they engage in 'invitation displays.'"

The conclusion to be drawn from these and other similar observations is inevitable. There must be some anatomical or physiological obstacle which prevents in most cases the completion of the interspecific copulations. Copulation is, of course, not entirely impossible, and if *pseudoobscura* ♂ stay sufficiently long with *persimilis* ♀ they will eventually inseminate most of them.

What the obstacle is that makes these interspecific matings so difficult has not yet been determined. A very careful study of the sexual armatures of males and females in the two species by Ferris and other workers has not yielded any apparent differences. There are obviously no "mechanical" barriers in the conventional meaning of the entomological literature. However, there may be invisible differences in the texture of the mucous membranes or in other physiological properties of the genital apparatus. Or else, the proper intromission of the phallus may require a high degree of receptivity ("coöperation") on part of the female. It is possible that the stimulation by the combined pre-copulatory display activities of the nonconspecific male is insufficient to produce the degree of receptivity in the female necessary for successful copulation. Observations gave the hard-to-prove impression that it was the female that was mainly responsible for the incompleteness of so many of the interspecific copulations. Further observations and experiments are required to solve this problem.

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TWO TYPES OF HETEROCHROMATIN IN DROSOPHILA NEBULOSA

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Introduction.—Euchromatin and heterochromatin are the two principal components of plant and animal chromosomes. Heitz[1] was the first to make a study of the relative quantities and distribution of these components in the mitotic chromosomes of *Drosophila*. Working with *Drosophila funebris*, he showed that in this species the heterochromatin is concentrated mostly in the sex chromosomes, making up the entire *Y* and half of the length of the *X*-chromosome which includes the centromere. The autosomes and the other half of the *X*-chromosome are formed mainly of euchromatin. In another work[2] the same author discussed the appearance of the heterochromatin in the salivary gland chromosomes. Here the heterochromatin forms the chromocenter and the bases of some of the chromosomes. Studying the salivary gland chromosomes of *D. virilis*[3] he concluded that the heterochromatin of this species is of two types, which he called, respectively, α-heterochromatin and β-heterochromatin. In the salivary gland nuclei, the α-heterochromatin forms a compact body, while

the β is more diffuse. While the α type forms only a part of the chromocenter, the β type forms parts of this structure and the bases of the chromosomes.

Painter[4] working with salivary gland chromosomes of *D. melanogaster* considered the chromocenter: "an amorphous mass of chromatic material to which all chromosomes or arms are attached." Muller and Prokofjeva[5] found in the heterochromatic part of the *X*-chromosome of *D. melanogaster* that "the appearance of this region in salivary gland material indicates that its chromonema has essentially the same structure as that of the active region. . . . " Bauer[6] working in Chironomus and in some species of Drosophila concluded that heterochromatic regions of salivary gland chromosomes are composed of the same number of chromonemata as the euchromatic portions, the difference between them being due to the structure of the single chromomeres. The euchromatin is formed by small completely stained dots (euchromomeres) while the heterochromatin is formed of large dots stained on the periphery (heterochromomeres).

Muller, Gershenson and Prokofjeva-Belgovskaya[7] showed that relatively long sections of the mitotic *X*-chromosome of *D. melanogaster* are reduced each to a single band in the salivary chromosome. Hinton,[8] also working in *D. melanogaster*, compared the heterochromatin of chromosome II in the mitotic and the salivary chromosomes. He showed that a heterochromatic section constituting $^1/_6$ of the left arm of the second chromosome in the mitotic metaphase is reduced to a single band in the salivary chromosome. Another region of about the same size, also in the second chromosome, gave rise to the remainder of the bulk of the heterochromatin of this salivary gland chromosome.

The present paper deals with the situation in *D. nebulosa*, in which there are two easily distinguishable types of heterochromatin. They have the same staining properties in the mitotic prophase and metaphase chromosomes, yet one of them is reduced in the salivaries to a few chromomeres only, while the other forms the bulk of the chromocenter. Since the two types of heterochromatin are confined to different chromosomes, the distinction between them is easy and more accurate than that in any other Drosophila species previously examined.

Material and Method.—D. nebulosa Sturtevant is the species used in the present work. It belongs to the *willistoni* group of the subgenus *Sophophora*. It has a wide distribution in the tropical Americas, from southern United States (Texas and Florida) to southern Brazil (São Paulo), including the West Indies.

Strains from Bertioga and Iporanga in the State of São Paulo, from Belem, State of Para, all in Brazil, and one strain from Del Rio, Texas, were used in this work. The last strain was kindly furnished by Prof. J. T. Patterson of the University of Texas.

All preparations were made by smearing the larval brain or salivary glands in acetic orcein.

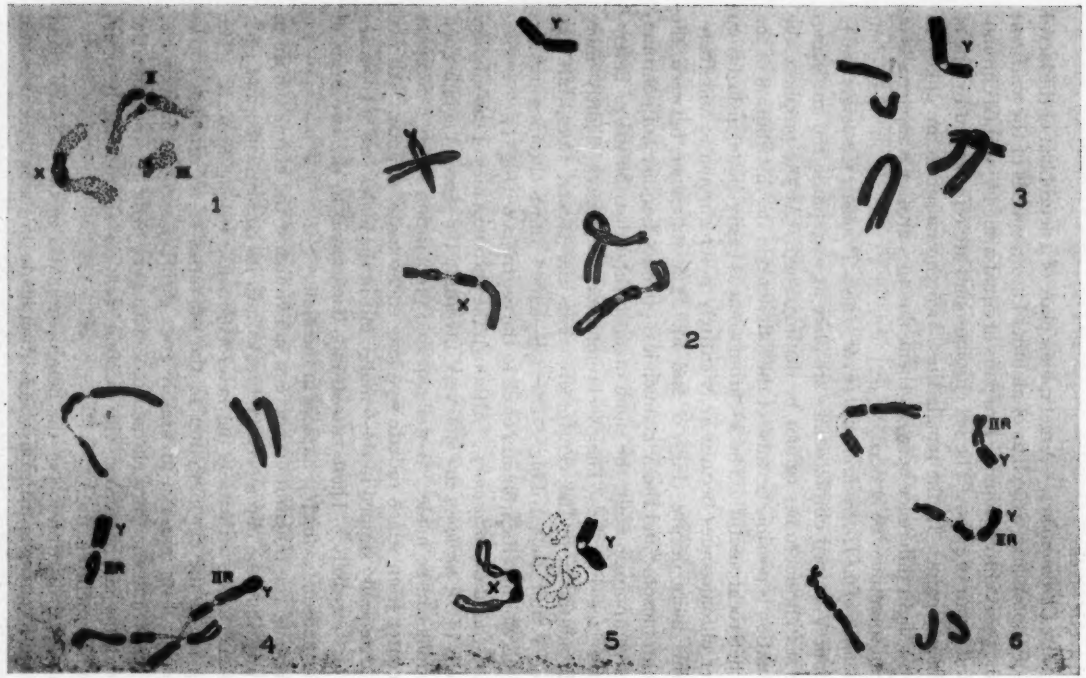
Metaphase Chromosomes.—A metaphase plate of a neuroblast of the larval ganglia of *D. nebulosa*, as well as of all other known species of the *willistoni* group, shows six chromosomes—two pairs of equal-armed V-shaped chromosomes and a pair of rods. The sex chromosomes are represented by one of the V-shaped pairs. In the female these two chromosomes are alike; in the male a slight difference between them, due to a greater compactness of the *Y*, can sometimes be noticed.

Distribution of the Heterochromatin in the Mitotic Prophase Chromosomes.—As the four V-shaped chromosomes are alike at metaphase the autosomes and the sex chromosomes cannot be distinguished. At the prophase the discrimination is possible because of the difference in the distribution of the eu- and heterochromatin. The *Y*-chromosome is easily distinguished from the other chromosomes because it is entirely heterochromatic and therefore stains more deeply (Figs. 2 and 5). The difference between the second chromosome and the *X* is much less obvious, because both chromosomes have approximately the same quantity and distribution of heterochromatin (see Fig. 1). The *X*-chromosome does have slightly more heterochromatin near the centromere than the second does, but the difference is not too striking. The rod-shaped third chromosome has a small piece of heterochromatin close to the centromere (Fig. 1).

Salivary Gland Chromosomes.—Although the chromosomes of the salivary gland cells of *D. nebulosa* are not very favorable for study, enough good preparations can be made. A well-smeared preparation shows a compact chromocenter from which radiate five long euchromatic strands. All five strands are equally stained in the females, but in the males two of them are stained less intensely. These two strands correspond to the two arms of the *X*-chromosome, which is haploid in the male.

By pressing the preparation one can individualize the chromosomes in such a way that the chromocenter breaks into its constituents, and each chromosome takes with itself the heterochromatin belonging to it. The breaking up of the chromocenter in the salivaries of *D. nebulosa* shows that almost all its material invariably goes with the second chromosome, leaving the *X* and the third chromosome with very little heterochromatin. The two arms of the second chromosome contain approximately equal pieces of heterochromatin.

The unequal distribution of the heterochromatin in the salivary gland chromosomes contrasts with the observation made on the heterochromatin in the mitotic prophase chromosomes, where the *X*-chromosome is provided with as much or more heterochromatin than the second, and where the third chromosome also has a small but easily noticeable heterochromatic section. This contrast is even more striking in the male. At mitotic



FIGURES 1-6

Figure 1—Early prophase in a neuroblast of a normal female of *Drosophila nebulosa*. Figures 2 and 5—Prophases in normal males. Figure 3—Prophase in Blade male. Figures 4 and 6—Prophases in Echinus males.

metaphase, the *Y*-chromosome differs from the *X* and the V-shaped autosomes only in its somewhat smaller size. At prophase, the *Y* is seen to be wholly heterochromatic while the *X* and the second have heterochromatic sections only in the vicinity of the centromeres. The heterochromatic section in the *X* is a little larger than that in the second. Finally, in the salivaries, the heterochromatin of the second chromosome makes up most of the chromocenter, while that of the *X* and the *Y* is reduced to only a few heterochromomeres.

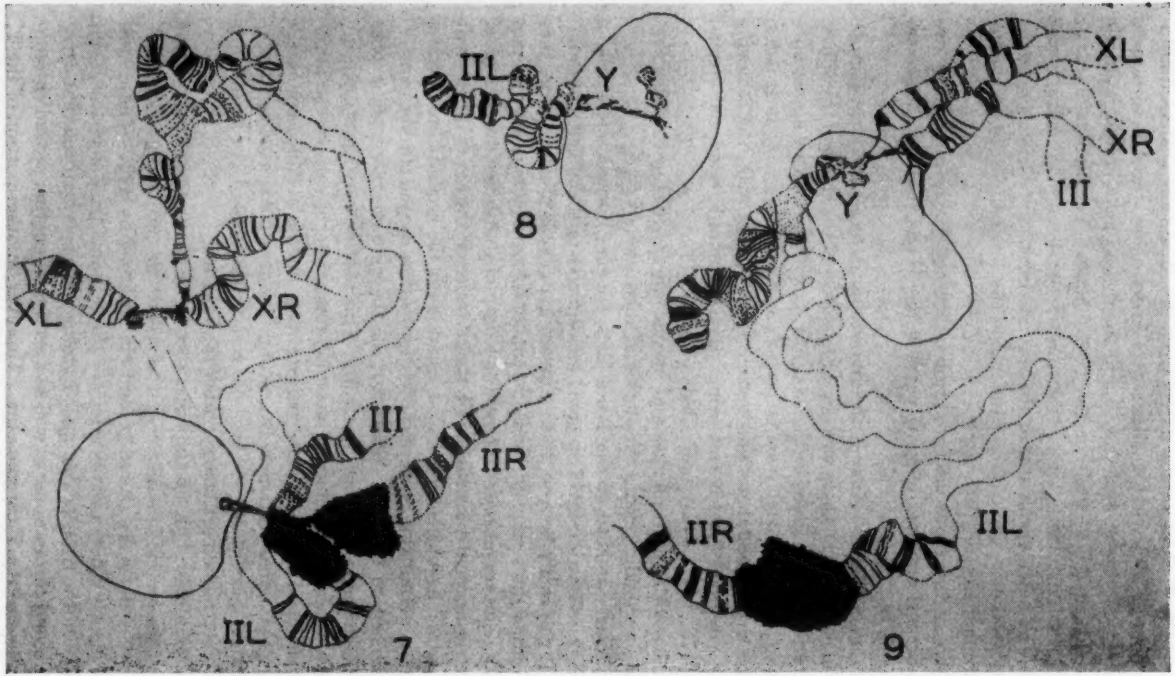
The proof of the above interpretation is afforded by the analysis of two reciprocal translocations involving parts of the *Y* and parts of the second chromosome.

Translocation Blade.—Among the mutations induced by x-rays in *D. nebulosa* males (4000 r), there was one which affected the shape of the wings. This mutation, which gives to the wing the sharp and narrow appearance called Blade, is transmitted from father to son, never appearing in females. Crosses between Blade males and normal females gave in F_1 the following results: normal body and normal wings ♀, 339; minute body and normal wings ♂, 173; normal body and Blade wings ♂, 181.

The males with minute bodies and normal wings are sterile. This suggests that these males carry a chromosomal aberration.

The mitotic prophase of the Blade flies appears normal, indicating a reciprocal translocation involving only small pieces of the affected chromosomes. It can easily be seen in the salivary gland cells of male larvae that the left limb of the second chromosome makes a loop touching the chromocenter at a point about four-fifths of the length of that limb from the base. When the chromocenter is crushed by the pressure on the cover slip, a great portion of the chromocenter goes, as usual, with the second chromosome. However, the terminal portion of the left limb of the second chromosome remains attached to the very small part of the chromocenter which goes with the *X*-chromosome (Figs. 7 and 9). This portion may show a pairing with its homologue in the normal second chromosome, as can be seen in the figures mentioned above, or else pairing may not be attained, in which case a small unpaired section of the second chromosome is attached to the chromocentral part of the *X*, and the rest of the second chromosome is free with its terminal part being thinner than the rest.

Some individuals show an unpaired section of the terminal part of the left limb of the second chromosome attached to the chromocenter of the *X* (Fig. 8) and a normally paired second chromosome as well. These individuals evidently carry a duplication for the terminal part of II-L. Their origin is due to the translocated male forming some spermatozoa that carry a normal second chromosome as well as a section of another second chromosome attached to the *Y*-chromosome. However, another portion of the *Y*, which is translocated onto the basal portion of the second chromosome, is



FIGURES 7–9

Figures 7 and 9—Chromocenter and the bases of the chromosome strands in the Blade translocation. Figure 8—The duplicating fragment of the left limb of the second chromosome in the Blade translocation.

absent in these males. This probably accounts for the sterility of the non-Blade males with minute bodies mentioned above; these males lack a portion of the *Y*-chromosome.

Translocation Echinus.—Among the offspring of the x-ray irradiated males giving rise to Blade, there appeared another mutation called Echinus. This mutant has rough eyes, with some ommatidia larger than others and with the rows of the ommatidia disarranged. The Echinus eyes are also somewhat smaller than normal. Like Blade, the Echinus mutation is transmitted from father to son only.

Crosses of Echinus males to wild-type females gave: normal ♀, 485; normal ♂, 2; Echinus ♂, 349.

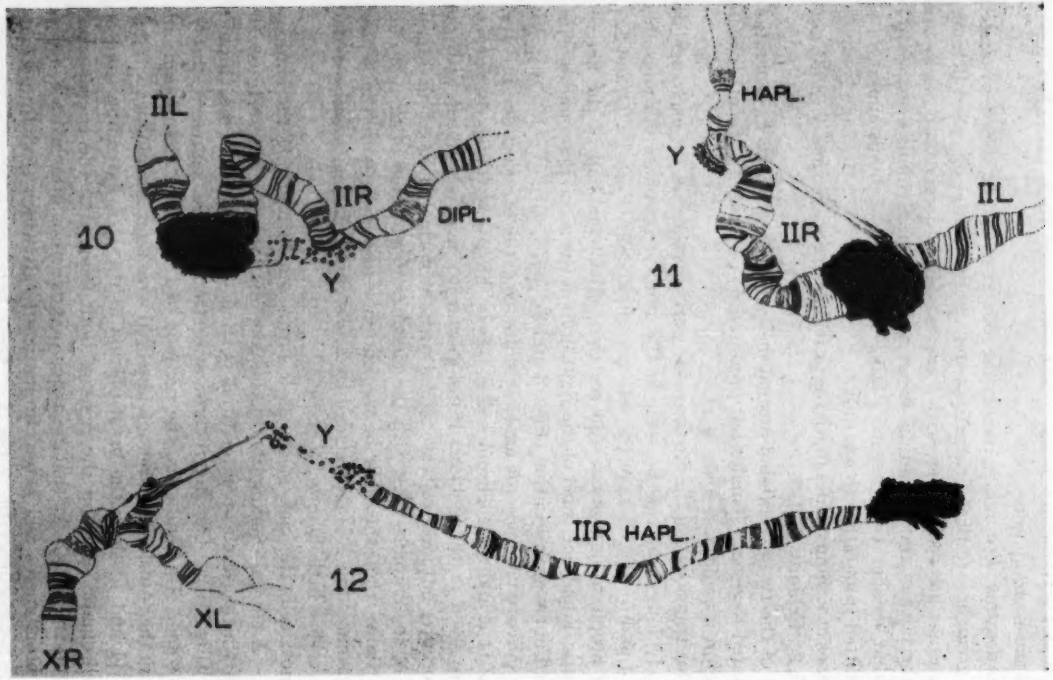
One of the two exceptional normal males observed in the F_1 generation was mated to several females but produced no offspring. The other male died before it could be tested.

In this translocation the chromosome parts involved in the exchange are relatively long, and can be seen in the mitotic prophase chromosomes (Figs. 4 and 6) as well as in the salivary gland cells (Figs. 10, 11 and 12). In the prophase figures one can see that almost one-half of the heterochromatic *Y* and one-third of the right limb of the second chromosome are involved in the translocation (Figs. 4 and 6).

The salivary chromosome analysis shows that the translocation involves parts of II-R and the *Y*-chromosome. As in the case of Blade, the parts of II-R involved in the Echinus translocation are now associated with the chromocenter but most cells are paired with the normal II-R. Individualization of the chromosomes with the aid of fragmentation of the chromocenter shows that the bulk of the chromocenter goes with the second chromosome; the *Y* is represented by a few chromomeres, and the *X* and III have very little heterochromatin.

In most cells the base of the II-R forms a loop touching the chromocenter. A careful examination of this loop discloses few heterochromatin strands which attach an interstitial portion of the II-R to the chromocenter (Fig. 10). The heterochromatic strands must represent the *Y*-chromosome. In some cells, the sections of the second chromosome involved in the translocation remain unpaired with their homologues. Thus, figures 11 and 12 show the basal portion of the II-R extending from its heterochromatic part (on the right of both figures) to the translocation break (on the left). At that breakage point there is attached a group of heterochromomeres and heterochromatic strands which tend to associate with the heterochromatin of the *X*. These heterochromomeres represent, then, the part of the *Y*-chromosome translocated to the II-R.

Discussion and Summary.—Taking into account the results of the previous workers and the data presented in this paper, it can be asserted that there exist at least two types of heterochromatin. One type shows



FIGURES 10–12

Chromocenter and the second chromosome in the Echinus translocation.

relatively little reduction in the salivary gland cells in proportion to the euchromatin in the mitotic chromosomes. Another kind of heterochromatin, which is not distinguishable from the first by its staining properties in the mitotic prophase, is reduced in the salivary gland cells to only a few heterochromomeres. The question of whether or not the two types of heterochromatin found in *D. nebulosa* correspond to the α and β types of Heitz[3] in *D. virilis* must be left open, because in this latter species it is difficult to localize these types of heterochromatin in the mitotic chromosomes and to compare them with the condition found in the salivary glands. It is necessary to point out that while Heitz[3] considered the α heterochromatin of *D. virilis* devoid of chromomeres, the heterochromatin of the *Y*-chromosome of *D. nebulosa* which might seem to resemble his α type is formed by typical Bauer's heterochromomeres.

The observations on the two types of heterochromatin found in *D. nebulosa* resemble most closely those of Muller, Gershenson and Prokofjeva-Belgovskaya[7] on the *X*-chromosome of *D. melanogaster* and those of Hinton[8] on the second chromosome of the same species.

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* Rockefeller Foundation Fellow.

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GENES WHICH DIVIDE SPECIES OR PRODUCE HYBRID VIGOR

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New light has been shed by recent genetic investigations on the old question of the origin of species. It is becoming increasingly clear that a species consisting of a group of organisms similar in appearance and freely interbreeding, may in the course of time become subdivided into groups

which no longer interbreed and which, becoming increasingly different in details of structure or function, constitute genuinely distinct species. The question is how do the specific differences originate. What is it that prevents interbreeding between groups of individuals destined to become distinct species?

Several methods of origin of specific differences have been demonstrated which involve change in the number of the chromosomes or in their structure. Such are (1) autopolyploidy, doubling of the chromosome number without qualitative change, converting a diploid species into a tetraploid; (2) allopolyploidy, union of gametes from different species followed by doubling of the new chromosome aggregate, insuring fertility of the hybrid under selfing or close inbreeding, but preventing backcrossing with either parent species; (3) change in the structure of individual chromosomes, as by inversion, deletion or interchange of parts between different chromosome pairs, resulting in partial sterility.

All such changes act as barriers to interbreeding between groups of individuals differing in one or more of the ways mentioned.

Lamprecht[1] maintains that a species barrier may also arise by a gene mutation which involves no gross chromosome change but only the coming into existence of a new dominant gene, the recessive allele of which causes sterility in certain combinations. He regards this as a more important if not more common mode of origin of specific differences than changes in chromosome numbers or gross chromosome structure.

It will be easier to grasp Lamprecht's idea if we have in mind the principle of a "killer" gene demonstrated in *Paramecium* by Sonneborn.[2]

He showed that a dominant "killer" gene K is carried without harmful effects in one race of *Paramecium aurelia*, and in other races of the same species a recessive allele k of the killer gene is carried also without harmful effects, though it makes the race "sensitive" to the killer product of the killer race. So that, if the two races are brought together, a killer substance produced in the killer race will cause the death of all individuals of the other "sensitive" race exposed to the action of killer substance.

Thus, the two races are prevented from interbreeding as a probable consequence of a single gene mutation.

To understand how such a situation may have arisen, let us suppose that in a self-fertilizing species a new dominant potentially lethal gene (K) arises, perhaps in the seemingly "inert" chromatin, in one member of a synaptic pair. The material opposite K in synapsis becomes its recessive allele k and may become sensitized to K. The two now separate and pass into different gametes and zygotes, each harmless except in association with the other in a newly formed zygote. Gamete K will pass into a zygote $K/-$, and this will produce progeny, some KK, others $K/-$, and still others $-/-$, fully normal. Similarly, gamete k will pass into a

zygote $k/-$, and produce descendants kk, $k/-$ and fully normal $-/-$. Homozygous lines KK and kk may ultimately be established by chance isolations, but they will be unable to cross with each other because of a lethal barrier, since K potential killer + k sensitizer produces a zygote K/k in which active killer substance is produced.

The situation just outlined is not purely hypothetical. It is exactly realized in crosses between certain species of Crepis investigated by Hollingshead.[3] In crosses of *C. tectorum* with *C. capillaris* and two other Crepis species, hybrid offspring were produced which failed to develop beyond the cotyledon state. It is assumed by Hollingshead that plants of *C. tectorum* which produce interspecific hybrids, all of which perish, are homozygous for a potentially lethal gene, which they transmit in all their gametes. Let us call the lethal gene K, and an assumed recessive allele and sensitizer of K in *C. capillaris*, k. Then in F_1 hybrids K (the potential lethal) combines with the sensitizer k to form a lethal K/k zygote.

Hollingshead was fortunate in finding other *C. tectorum* plants which in crosses with *C. capillaris* produced only 50 per cent of lethal seedlings, the other 50 per cent being viable and fully fertile in F_1 and subsequent generations.

We may assume, as Hollingshead does, that such a *C. tectorum* plant was heterozygous for the lethal gene. Its constitution may be expressed as $K/-$ (*not* K/k,) since no sensitizer action was shown.

The 50 per cent of viable F_1 hybrids, would derive k, the sensitizer allele of K, from their *C. capillaris* parent, but this would be harmless in the absence of K. Their genetic formula would be $k/-$ since they would carry only a single dose of k. But in later generations their descendants under selfing would become k/k, $k/-$ and $-/-$. In reality Hollingshead in her experiments found all *C. capillaris* plants tested to be homozygous k/k for the assumed recessive allele and sensitizer of K, since all gave the lethal reaction when combined with ordinary K gametes of *C. tectorum*.

Hollingshead found that in crosses with two other species of Crepis, no lethal action of the potentially lethal gene of *C. tectorum* was found, the F_1 hybrids all being viable. It is obvious that such species do not carry the assumed sensitizer k. Their formula as regards the genetic locus occupied by K of *C. tectorum* and k of *C. capillaris* being $-/-$. This would correspond with the constitution of ancestral species of Crepis before the advent of the mutation K/k, as also with that of $-/-$ derivatives of $k/-$ zygotes.

In the light of the foregoing, let us now examine a case assumed by Lamprecht to fall in a category of species separating mutations.

Two species of cultivated beans, *Phaseolus vulgaris* and *P. multiflorus*, were found, when artificially crossed, to produce vigorous and fertile F_1 hybrids. In F_2 all characters, in which the two species differ, recombine freely in viable zygotes, with a single exception. The exception concerns a

gene *Epi* of *P. vulgaris*, and its allele *Hyp* of *P. multiflorus*. *Epi* is responsible for the epigean position of the cotyledons in seedlings of the common bean germinated in soil; *Hyp* is responsible for the hypogean position of the cotyledons in germinating seeds of *P. multiflorus*. F_1 hybrids show an intermediate character.

When *P. vulgaris* is the mother plant in the cross, the F_1 plant has vulgaris plasma. In that plasma gametogenesis is entirely normal, segregating into carriers of *Epi* and *Hyp*, respectively. The former enter into the production of fully fertile plants being in their native plasma, but the latter fail to develop further; for they are in foreign plasma which apparently does not give them the normal stimulus necessary to fruitful development.

In the reciprocal cross, in which the F_1 plant derives its plasma from *C. multiflorus*, the *Hyp* gene produces fruitful offspring (being in its native plasma) but carriers of the *Epi* gene fail to develop further.

It thus appears that *Epi* and *Hyp* are both dominant species-specific alleles at a common genetic locus, occurring normally in different species. F_1 hybrids show an intermediate character, thus demonstrating that both are present in an active state. As regards this gene pair, F_1 is *Epi/Hyp* in formula. But each allele in its native plasma acts as a *sensitizer* of that plasma to the other allele, which thus becomes sterilized. In mother plants having *Epi* plasma, *Hyp* gametes fail to develop; and in mother plants having *Hyp* plasma, *Epi* gametes fail to develop.

It is possible that the puzzling phenomenon of hybrid vigor may find its explanation along similar lines. Jones,[4] has recently shown that heterosis may be found in the superior growth energy of a hybrid between two inbred lines of maize, related as mother and daughter strains, and differing from each other in a single gene pair, the dominant allele being found in the mother strain, the recessive in the daughter strain. On the evidence presented by Jones, it can be only the stimulating action resulting from the union of the dominant with the recessive allele of that single pair of genes which is accountable for the increased growth energy, superior to that of either parent strain.

Let us suppose that in the mother strain a new dominant gene *A* has made its appearance in the unorganized chromatin of a single chromosome, and that it has sensitized (after the manner of anaphylaxis) the chromatin which lay opposite that locus in the other member of the chromosome pair, thus creating a recessive allele, *a*.

The two alleles will pass into different gametes and may later become components of different homozygous strains, *AA* in the mother strain, *aa* in the mutant daughter strain. Each strain will maintain its distinctive morphological character, so far as this gene pair creates a distinction. Each also will manifest a characteristic growth energy. But when the two are

crossed, an increased growth energy is shown by the hybrid, a hybrid, be it remembered, as Jones clearly shows, in only a single gene pair, *Aa*.

This case is similar to that of the killer mutation of Sonneborn, except that the action induced in the dominant gene by its sensitized recessive, instead of being harmful is in this case beneficial.

Jones points out that in maize and other plants not all heterozygous unions of a dominant with its recessive allele result in increased growth energy. In the production commercially of hybrid corn, not all crosses of inbred lines result in hybrid vigor. In such cases no sensitizer action between a dominant and its recessive allele seems to have occurred. The alleles instead of being A and a sensitized recessive allele a, would seem to be A and an absence of a sensitized allele. In the cases reported by Jones as showing heterosis, the zygote may be assumed to be A/a in constitution. In cases not showing hybrid vigor, it may possibly be $A/-$, the $-$ representing the condition of that locus in the chromatin prior to the origin of the A gene.

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PRIME NUMBER OF OPERATORS IN SETS OF CONJUGATES

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Suppose that the order of the group G is p^m, p being a prime number, and that each set of non-invariant conjugate operators of G contains p operators. Each of the non-invariant operators of G is then invariant under a subgroup of index p under G and this subgroup is therefore an invariant subgroup of G and includes the given p conjugate operators as well as the central of G. This central is contained in an Abelian invariant subgroup of G whose order is p times the order of this central and is itself contained in an invariant subgroup of G whose order is p^2 times the order of the central of G. This subgroup H_1 transforms its own operators in exactly the same way as these operators are transformed under G itself and contains the same central as G contains since each non-invariant operator of G is assumed to have exactly p conjugates under G.

The group of inner isomorphisms of G can be represented as an intransitive permutation group each of whose transitive constituents is of degree p. These constituents must also be of order p since the order of every operator of G is a power of p. The group of inner isomorphisms of G is therefore an Abelian group all of whose operators besides the identity are of order p. It will be proved that in this Abelian group of order p^n, n is always an even number. In the given subgroup H_1 n is 2 and the commutator subgroup is of order p because this commutator subgroup is in the central of G, and if its order would exceed p then G would contain a set of more than p conjugate operators, which is contrary to the hypothesis. Since the group of inner isomorphisms of G is Abelian, the commutator subgroup of G is included in the central of G.

It was noted near the close of the first paragraph that the operators of H_1 are transformed under H_1 in the same ways as they are transformed under G. Hence the operators of G which are not also found in H_1 include at least one operator s_1 which is commutative with every operator of H_1 and has its pth power in H_1. If the order of G exceeds p^2 times the order of the central of G this will clearly be also true of the operators which are found in G but do not appear among the operators of a larger invariant subgroup of G. In particular, the subgroup H_1 can be extended so as to obtain a group whose order is p^4 times the order of the central of G and which is invariant under G and transforms its non-invariant operators in the same ways as these operators are transformed under G and involves the same central as G involves.

To prove that this extended group involves the same commutator subgroup as H_1 involves we may assume that its commutator subgroup involves two independent generators t_1, t_2 and prove that this leads to a contradiction. It was noted above that the commutator subgroup of G cannot involve any operator of order p^2 since the commutators of G appear in its central. We may assume that the two operators s_1 and s_2 of H_1 give rise to the commutator t_1 and that the two operators s_3 and s_4 of the given subgroup H_2 whose order is p^2 times the order of H_1 give rise to the commutator t_2. Moreover, it may be assumed that both of the operators s_3 and s_4 are commutative with each of the two operators s_1 and s_2. It would then follow that the product s_1s_3 would have more than p conjugates under G; which is contrary to the hypothesis. Hence it results that *if in a group of order p^m, p being a prime number, all the non-invariant operators have p conjugates the commutator subgroup is of order p and is in the central of G.*

To prove that n, the index of the power of p which is equal to the order of the group of inner isomorphisms of G, is always even it is only necessary to observe that in constructing G in the manner noted above, the order of the resulting group which transforms its operators in the same ways as the operators are transformed under G proceeds by p^2 times the order of the

group already found whose operators have this property. For instance, the order of H_2 is p^2 times the order of H_1 and the order of H_1 is p^2 times the order of the central of G. The group obtained by forming the direct product of G and any Abelian group clearly has the same group of inner isomorphisms as G has.

The fact that every set of non-invariant conjugate operators of a group is assumed to contain a prime number of operators by itself imposes a strong condition on a group but it does not necessarily restrict the order of the group to be a power of a single prime number. This results directly from the fact that if the order of a non-cyclic group is the product of two distinct prime numbers then the larger of these numbers diminished by unity is divisible by the smaller and each operator of the group besides the identity has a prime number of conjugates under the group. This is also true of the direct products of an arbitrary Abelian group and this non-cyclic group. A necessary and sufficient condition that the index of every proper subgroup of a group is a prime number is obviously that the order of the group is the product of two prime numbers.

If we assume that every set of non-invariant operators of G is composed of a prime number of operators but do not assume that the order of G is a power of a prime number then the operators which are commutative with a given non-invariant operator s of G constitute a subgroup of prime index under G which is not necessarily invariant under G. If this subgroup is assumed to be Abelian in every case then two such distinct subgroups will have a cross-cut which is in the central of G and hence is invariant under G. The quotient group of G with respect to this invariant subgroup has an order which is the product of two prime numbers. Hence it results that *when all the non-invariant operators of a group appear in sets of conjugates such that each set is composed of a prime number of operators and all the operators which are commutative with a given non-invariant operator constitute an Abelian group then the order of the central quotient group of the group is the product of two prime numbers.*

Since a group whose order is the product of two prime numbers always involves an invariant subgroup of prime index it has been proved that if all the non-invariant operators of a group appear in sets of conjugates which are such that the number of the operators in each set of conjugates is a prime number and if the total number of the operators which are commutative with a non-invariant operator always constitute an Abelian subgroup of prime index under the group then the group contains an invariant subgroup of prime index under the group. It may be noted that in the special system of non-cyclic groups whose orders are the products of two distinct prime numbers and in the direct products of such groups and arbitrary Abelian groups these conditions are satisfied. It should be emphasized that when each of two distinct subgroups is of prime index under

a given group then it is not possible for one of them to be contained in the other.

If the operators which are commutative with one of the non-invariant operators of one of the groups under consideration do not always constitute an Abelian group then the prime number of the conjugates for each of its sets of conjugate operators of its non-invariant operators must be the same and hence these groups were considered before in these PROCEEDINGS, **32**, 53–56 (1946) under a somewhat more general title, which has much in common with the present article. This article aims to clarify some of the common points as well as to extend the types of the groups under consideration. The two articles together aim to consider fundamental questions of all the groups in which all the non-invariant operators appear in sets of conjugates such that each set involves a prime number of operators. It results that *the number of different primes involved in the same group cannot exceed two* and that when there are two such primes the larger diminished by unity is divisible by the smaller. The fact that there cannot be more than two such prime numbers in a given group seems to deserve special emphasis.

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*COMPARISON OF UNION-PRESERVING AND CONTACT TRANSFORMATIONS**

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1. Union-Preserving and Contact Transformations.—In our Bulletin paper of 1944, we studied transformations, in space, of curve-elements of order n, where n is 2 or more, into lineal-elements.[1] The general class of union-preserving transformations, where the point of the transformed lineal-element actually depends on the derivatives of order n, is defined by a single directrix equation

$$\Omega(X, Y, Z, x, y, z, y', z', \ldots, y^{(n-2)}, z^{(n-2)}) = 0, \qquad (U)$$

involving derivatives of order $(n - 2)$.

As an example, consider the problem of finding the locus of the center of spherical curvature of a twisted curve in space. There is deduced a union-preserving transformation, defined by the directrix equation

$$(X - x) + y'(Y - y) + z'(Z - z) = 0, \qquad (E)$$

from curve-elements of third order into lineal-elements.

Sophus Lie studied transformations of surface-elements (x, y, z, p, q) into surface-elements. The general class of contact transformations in this classic theory is defined by the directrix equation

$$\Omega(X, Y, Z, x, y, z) = 0, \qquad \text{(L)}$$

or by a pair or a triplet of such directrix equations. All the contact transformations of surface-elements of higher order into elements of same order are simply the extensions of these (as proved by Backlund).

If, and only if, $n = 2$, the directrix equation (U) does not contain any derivatives. The union-preserving transformations of curve-elements of second order into lineal-elements are defined by a single directrix equation which in this case is of the form (L). Thus the directrix equation (L) defines a contact transformation Γ of surface-elements according to Lie, and a union-preserving transformation T from second-order curve-elements into lineal-elements according to our new theory.

In the present article, we shall give a comparison between the contact transformation Γ and the union-preserving transformation T, both of which are defined by the same directrix equation (L). This phenomenon occurs in spaces of three or more dimensions. In the plane, we have already stated that Γ and T are identical[2] when there are no derivatives in Ω.

2. The Union-Preserving Transformation T from Curve-Elements of Second Order into Lineal-Elements.—The point of the transformed lineal-element (X, Y, Z, Y', Z') is obtained by solving for (X, Y, Z), the three equations[3]

$$\left.\begin{aligned} \Omega(X, Y, Z, x, y, z) = 0, \qquad \Omega_x + y'\Omega_y + z'\Omega_z = 0, \\ \Omega_{xx} + y'^2\Omega_{yy} + z'^2\Omega_{zz} + 2y'z'\Omega_{yz} + 2z'\Omega_{xz} + 2y'\Omega_{xy} + y''\Omega_y + z''\Omega_z = 0. \end{aligned}\right\} (1)$$

The direction is found by solving for (Y', Z'), the two extra equations

$$\left.\begin{aligned} \Omega_X + Y'\Omega_Y + Z'\Omega_Z = 0, \\ (\Omega_{xX} + y'\Omega_{yX} + z'\Omega_{zX}) + Y'(\Omega_{zY} + y'\Omega_{yY} + z'\Omega_{zY}) + Z'(\Omega_{zZ} + \\ y'\Omega_{yZ} + z'\Omega_{zZ}) = 0. \end{aligned}\right\} (2)$$

We consider briefly the transforms under our union-preserving correspondence T, of a lineal-element (x, y, z, y', z') and of a planar-element (x, y, z, p, q).

In the first place, it is noticed that a single point (x, y, z) corresponds to a surface Σ, defined by the equation (L). The lineal element (X, Y, Z, Y', Z') is that element on Σ which is on the edge of regression of the one-parameter family of surfaces which correspond by (L) to the points of the curve: $y = y(x)$, $z = z(x)$.

A lineal-element (x, y, z, y', z') corresponds by (1) to a curve C, which is given by the first two of equations (1). This curve C is the locus of ultimate intersections of the two surfaces which correspond to two neighboring

points. Of course, the point of the transformed lineal-element (X, Y, Z, Y', Z') is on this curve C.

A planar-element (x, y, z, p, q) may be considered as consisting of the point (x, y, z) and the osculating plane of the second order curve-element. Thus $(p, q, -1)$ are the direction numbers of the binormal, so that $z' = p + qy'$ and $z'' = qy''$. In general, it is shown that a fixed planar element corresponds to the whole surface Σ. The complete correspondence is a single lineal-element at each point of Σ. That is, a planar-element corresponds to ∞^2 lineal-elements, all tangent to the surface Σ.

By (1), it can be shown that *an arbitrary planar element can correspond to a single planar element if, and only if, each surface which is associated to any point* (X, Y, Z) *by* (L), *is a plane.*

A second union-preserving transformation can be defined by (L) from the (X, Y, Z)-space to the (x, y, z)-space. If this also converts a planar element into a planar element, then our correspondence must be a correlation.

3. The Lie Contact Transformation Γ.—The point of the transformed planar-element (X, Y, Z, P, Q) is found by solving for (X, Y, Z) the three equations[3]

$$\left.\begin{aligned} \Omega(X, Y, Z, x, y, z) &= 0, \\ \Omega_x + p\Omega_z = 0, \qquad \Omega_y + q\Omega_z &= 0. \end{aligned}\right\} \tag{3}$$

The direction of the normal to the plane is obtained by solving for (P, Q) the two equations

$$\Omega_X + P\Omega_Z = 0, \qquad \Omega_Y + Q\Omega_Z = 0. \tag{4}$$

By (1) and (3), it is seen that the point of the transformed planar-element under the contact transformation Γ is on the curve C defined by the first two of equations (1). Of course, this is the characteristic point on the surface Σ of the two-parameter family of surfaces which correspond by (L) to the points of the surface: $z = z(x, y)$.

This characteristic point is different, in general, from the point of the transformed lineal-element under our union-preserving transformation T. *The two transformations* T *and* Γ *are effectively identical if, and only if, each surface which is associated to any point* (X, Y, Z) *by* (L), *is a plane.*

Let T' denote the union-preserving transformation which by (L) carries any second order curve-element $(X, Y, Z, Y', Z', Y'', Z'')$ into a lineal-element (x, y, z, y', z'). In general, this is not the inverse of the original union-preserving transformation T. Also let Γ' be the inverse of the contact transformation Γ.

The union-preserving transformations T *and* T' *will coincide with the contact correspondences* Γ *and* Γ', *respectively, only for the case of a correlation.*

We shall illustrate our general theory with some noteworthy examples.

4. *Examples.—Polarity* with respect to the sphere: $x^2 + y^2 + z^2 = a^2$ is given by the directrix equation

$$xX + yY + zZ = a^2. \tag{5}$$

In this case, it is shown easily that not only T and Γ, but also T' and Γ', are identical.

Next consider the directrix equation

$$(X - x)^2 + (Y - y)^2 + (Z - z)^2 = a^2. \tag{6}$$

This carries each point (x, y, z) into a sphere with radius a and with center at the original point.

The contact transformation Γ defined by (6) is known as *dilatation*. It moves each planar-element in a direction perpendicular to itself through a constant distance a. Any surface is turned into a parallel surface and we have the theorems of Gauss.

But the same simple equation (6) defines a new transformation T according to our theory which we term *quasi-dilatation*.

We thus obtain a new theory of quasi-parallel curves in space by means of the union-preserving transformation T whose directrix equation is (6).

This correspondence T is given by the equations

$$\left.\begin{aligned} &(X - x)^2 + (Y - y)^2 + (Z - z)^2 = a^2, \\ (X - x) + y'(Y - y) + z'(Z - z) = 0, \quad &y''(Y - y) + z''(Z - z) = 1 + y'^2 + z'^2, \\ (X - x) + Y'(Y - y) + Z'(Z - z) = 0, \quad &1 + y'Y' + z'Z' = 0. \end{aligned}\right\} \tag{7}$$

This transformation T is constructed geometrically in the following manner. Draw the circle of curvature to any second order curve-element. Let B be the straight line through the center of this circle and orthogonal to the osculating plane. The corresponding points (X, Y, Z) are the intersections of this line B and the sphere (6). The directions (Y', Z') are perpendicular to the radius vector connecting the corresponding points (x, y, z) and (X, Y, Z) and the tangent direction of the original curve-element. Thus these three directions are mutually orthogonal.

Under our new theory of quasi-parallel curves, we find that *the quasi-parallel of a helix is a helix*. The quasi-parallel of a plane curve is on the cylinder with generators orthogonal to the plane of the original curve and passing through its evolute. In general, the new quasi-parallel of a plane curve is gauche.

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[1] "Union-Preserving Transformations of Space," *Bull. Am. Math. Soc.*, **50**, 98–107 (1944).

[2] "Union-Preserving Transformations of Differential Elements," these PROCEEDINGS, **29**, 271–275 (1943). Also "A Generalized Theory of Contact Transformations," *Revista de matematicas del Universidad de Tucuman (Argentina)*, **4**, 81–90 (1944). A typical ex-

ample in this theory is Huyghen's transformation from any curve to its evolute. Our whole theory may be regarded as a natural extension of Huygen's discussion of evolutes, involutes and wave propogation; and optical aspects will be considered elsewhere.

[3] In both the Lie theory and the new theory of the present paper, it is of course assumed that the directrix equation actually represents a triple infinity of surfaces in the (x, y, z) space. Hence certain functional determinants do not vanish.

AN ERGODIC THEOREM

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The purpose of this note is to state and sketch the proof of a theorem of the ergodic type (Theorem 1) and to discuss and clarify the relations among Theorem 1 and the results of Birkhoff-Khintchine, Hopf and Hurewicz. It turns out that Theorem 1 (or, rather, its easy consequence, Theorem 6) includes as a special case the hitherto most inclusive result (namely the theorem of Hurewicz, which appears below as Theorem 7). The extent to which Theorem 6 is a generalization of the theorem of Hurewicz is exactly the same as the extent to which Hopf's result is a generalization of Birkhoff's. Both the statement and the proof of Theorem 1 are more natural, and in several details more simple, than those of Theorem 7. In section 5 the important question of the deducibility of Theorem 1 from the known results on measure preserving transformations is treated.

1. Let X be a measure space which is the union of countably many measurable sets of finite measure, and let T be a one to one transformation of X onto itself. The transformation T is *measurable* if both T and T^{-1} send measurable sets into measurable sets; it is *non-singular* if both T and T^{-1} send sets of measure zero into sets of measure zero. If T is measurable and non-singular then the Radon-Nikodym theorem yields the existence of a measurable function $\omega_n(x)$ (which may be assumed to be everywhere positive) such that for every measurable set E, $m(T^nE) = \int_E \omega_n(x)dm(x)$. For any real valued function $q(x)$ and any positive integer n the notation $q^n(x)$ will be used for the sum $q^n(x) = \sum_{i=0}^{n-1} q(T^ix)\omega_i(x)$.

THEOREM 1. *If T is measurable and non-singular, $f(x)$ is integrable, $g(x)$ is non-negative, measurable, and such that $\lim_{n\to\infty} g^n(x) = \infty$ almost everywhere, then $f^n(x)/g^n(x)$ converges alomst everywhere to a finite limit.*

The proof of Theorem 1 appears in section 4.

Observe that $f^n(x)/g^n(x)$ may be considered as the ratio of two expres-

sions of the form $(\sum_{i=0}^{n-1} q(T^i x)\omega_i(x))/(\sum_{i=0}^{n-1}\omega_i(x))$. Such an expression is the average of the first n terms of the sequence $\{q(T^i x)\}$ weighted by the first n terms of the sequence of densities $\{\omega_i(x)\}$. If it were permissible to let $g(x)$ be identically equal to 1 (as it is, under certain conditions discussed below) then, for this g, f^n/g^n itself would be a weighted average.

If T is measure preserving then $\omega_i(x) = 1$ for every i and x, and for $g(x) = 1$, $g^n(x) = n$. Consequently in this most important special case Theorem 1 reduces to the classical ergodic theorem.

THEOREM 2 (Birkhoff[1]-Khintchine[2]). *If T is measure preserving and $f(x)$ is integrable then $(\sum_{i=0}^{n-1} f(T^i x))/n$ converges almost everywhere to a finite limit.*

2. The transformation T is *incompressible* if for every measurable set E, $TE \subseteqq E$ implies $m(E - TE) = 0$. The function $g(x)$ is *invariantly positive* if it is non-negative, measurable, and such that there is no invariant set of positive measure on which it vanishes. Observe that if g is measurable and almost everywhere positive (in particular if $g(x) = 1$) then g is *a fortiori* invariantly positive.

THEOREM 3. *If T is measurable and non-singular, $g(x)$ is non-negative and measurable, and $\lim_{n\to\infty} g^n(x) = \infty$ almost everywhere, then g is invariantly positive. Conversely if T is measurable, non-singular, and incompressible, and if g is invariantly positive, then $\lim_{n\to\infty} g^n(x) = \infty$ almost everywhere.*

Proof. If $\lim_{n\to\infty} g^n(x) = \infty$ almost everywhere and A is a measurable invariant set such that for $x \in A$, $g(x) = 0$, then for $x \in A$, $\lim_{n\to\infty} g^n(x) = 0$. It follows that $m(A) = 0$, so that g is invariantly positive.

Suppose, conversely, that g is invariantly positive and define $\mu(E) = \int_E g(x)dm(x)$. Let k be any positive number and let E be any measurable set of finite measure on which $\sum_{i=0}^{\infty}(T^i x)\omega_i(x) < k$. Then $\infty > km(E) \geqq \int_E \sum_{i=0}^{\infty} g(T^i x)\omega_i(x)dm(x) = \sum_{i=0}^{\infty}\int_{T^i E} g(x)dm(x) = \sum_{i=0}^{\infty}\mu(T^i E)$. If $F = \bigcup_{n=0}^{\infty} T^n E$ then, since $TF \subseteqq F$ and T is incompressible, it follows that the sets F, TF, T^2F, ... are all equal except for a set of m measure zero and therefore except for a set of μ measure zero. Hence

$$\mu(F) = \mu(\bigcap_{k=0}^{\infty} T^k F) = \mu(\bigcap_{k=0}^{\infty}\bigcup_{i=k}^{\infty} T^i E) \leqq \sum_{i=k}^{\infty}\mu(T^i E),$$

and it follows that $\mu(F) = 0$. The incompressibility of T together with its non-singularity implies that the invariant set $F^* = \bigcup_{-\infty}^{\infty} T^n F$ differs from F on at most a set of measure zero, whence

$$0 = \mu(F) = \int_F g dm = \int_{F^*} g dm.$$

Since g is invariantly positive, it follows that $m(E) \leqq m(F) \leqq m(F^*) = 0$. Since, by the hypothesis on the space X, the set of points x at which $\lim_{n\to\infty} g(x) < \infty$ is the union of countably many sets such as E, the proof of the theorem is complete.

Theorems 1 and 3 together imply the following two results.

THEOREM 4. *If T is measurable, non-singular, and incompressible, $f(x)$ is integrable, and $g(x)$ is invariantly positive, then $f^n(x)/g^n(x)$ converges almost everywhere to a finite limit.*

THEOREM 5 (Hopf[3]). *If T is measure preserving and incompressible, $f(x)$ is integrable, and $g(x)$ is invariantly positive, then $(\sum_{i=0}^{n-1} f(T^i x))/(\sum_{i=0}^{n-1} g(T^i x))$ converges almost everywhere to a finite limit.*

Hopf himself stated this result only under the condition that $g(x)$ is integrable and almost everywhere positive. The present generality is sometimes technically useful.

3. The results of section 2 have applications even to the possibly singular case.

THEOREM 6. *If T is measurable and incompressible, $f(x)$ is integrable, and $g(x)$ is measurable and almost everywhere positive, and if $h_n(x)$ is defined, by means of the Radon-Nikodym theorem, by the relation $\sum_{i=0}^{n-1} \int_{T^i E} f(x)dm(x) = \int_E h_n(x)d\mu^n(x)$, where $\mu^n(E) = \sum_{i=0}^{n-1} \int_{T^i E} g(x)dm(x)$, then $h_n(x)$ converges almost everywhere to a finite limit.*

Proof. Assume first that $m(X) < \infty$. If $\mu^n(E) = 0$ then, since g is positive, $m(T^i E) = 0$ and therefore $\sum_{i=0}^{n-1} \int_{T^i E} f(x)dm(x) = 0$, so that the Radon-Nikodym theorem may indeed be applied. Let $\{c_n : n = 0, \pm 1, \pm 2, \ldots\}$ be a sequence of positive constants whose sum is 1 and write $\bar{m}(E) = \sum_{-\infty}^{\infty} c_n m(T^n E)$. With respect to the finite measure $\bar{m}$ the transformation T is non-singular and incompressible. To prove the latter statement, observe first that m and $\bar{m}$ are identical on invariant sets. If E is a measurable set for which $TE \subseteq E$ then $T^{n+1}E \subseteq T^n E$, so that

$$\bar{m}(E - TE) \leq \bar{m}(\bigcup_{-\infty}^{\infty}(T^n E - T^{n+1}E)) = m(\bigcup_{-\infty}^{\infty}(T^n E - T^{n+1}E)) = 0,$$

and therefore T is indeed incompressible with respect to $\bar{m}$. Since $\bar{m}(E) = 0$ implies $m(E) = 0$, a non-negative measurable function $p(x)$ may be found so that for every measurable set E, $m(E) = \int_E p(x)dm(x)$; the fact that m and $\bar{m}$ are identical on invariant sets implies that $p(x)$ is invariantly positive with respect to $\bar{m}$. Since, finally, $\int_X f(x)dm(x) = \int_X f(x)p(x)d\bar{m}(x)$, it follows that $f(x)p(x)$ is integrable with respect to $\bar{m}$, and consequently that Theorem 4 may be applied to the transformation T, the measure $\bar{m}$, the integrable function $\bar{f}(x) = f(x)p(x)$, and the invariantly positive function $\bar{g}(x) = g(x)p(x)$. The conclusion is that $\bar{f}^n/\bar{g}^n$ converges to a finite limit almost everywhere with respect to $\bar{m}$ and hence almost everywhere with respect to m. The proof of Theorem 6 will be completed by calculating h_n and observing that it is equal to $\bar{f}^n/\bar{g}^n$ almost everywhere. Since

$$\begin{aligned}\mu^n(E) &= \sum_{i=0}^{n-1} \int_{T^i E} g(x)dm(x) = \sum_{i=0}^{n-1} \int_{T^i E} \bar{g}(x)d(\bar{m}x) \\ &= \sum_{i=0}^{n-1} \int_E \bar{g}(T^i x)\omega_i(x)d\bar{m}(x) = \int_E \bar{g}^n(x)d\bar{m}(x),\end{aligned}$$

it follows that

$$\int_E \bar{f}^n(x)d\bar{m}(x) = \sum_{i=0}^{n-1}\int_E \bar{f}(T^ix)\omega_i(x)d\bar{m}(x) = \sum_{i=0}^{n-1}\int_{T^iE}\bar{f}(x)d\bar{m}(x) = \sum_{i=0}^{n-1}\int_{T^iE}f(x)dm(x) = \int_E h_n(x)d\mu^n(x) = \int_E h_n(x)\bar{g}^n(x)d\bar{m}(x);$$

the equality of the first and last members of the last written chain of equations, for all measurable sets E, implies the desired result.

If $m(X) = \infty$, let m' be a finite measure whose vanishing is equivalent to that of m. (The existence of such an m' is a consequence of the assumption that X is the union of countably many sets of finite measure.) Then $m(E) = \int_E k(x)dm'(x)$ with a positive measurable function $k(x)$; write $f' = kf$, $g' = kg$, and apply the result just proved to f', g', m'. The fact that for any measurable set E, $\int_E f'dm' = \int_E fdm$ and $\int_E g'dm' = \int_E gdm$ concludes the proof of Theorem 6.

If $g(x) = 1$, Theorem 6 may be stated as follows.

THEOREM 7 (Hurewicz[4]). *If T is measurable and incompressible and $f(x)$ is integrable, and if $h_n(x)$ is defined by the relation $\sum_{i=0}^{n-1}\int_{T^iE}f(x)dm(x) = \int_E h_n(x)dm^n(x)$, where $m^n(E) = \sum_{i=0}^{n-1}m(T^iE)$, then $h_n(x)$ converges almost everywhere to a finite limit.*

4. In this section the proof of Theorem 1 is outlined. Since the proof uses no essentially novel methods and makes some very minor simplifications only, as compared with the corresponding proofs of Khintchine, Hopf and Hurewicz, a more than customarily condensed telegraphic style will be employed.

(*A*) Since $\int_E \omega_{i+j}(x)dm(x) = m(T^{i+j}E) = \int_{T^jE}\omega_i(x)dm(x) = \int_E \omega_i(T^jx)\omega_j(x)dm(x)$, the relations $\omega_{i+j}(x) = \omega_i(T^jx)\omega_j(x)$ may be assumed to hold for every x. It follows that for $1 \leqq i < j = 1, 2, 3, \ldots$, $q^j(x) = q^i(x) + \omega_i(x)q^{j-i}(T^ix)$ for every real valued function $q(x)$.

(*B*) If $q(x)$ is measurable and either its positive or its negative part is integrable (so that $\int_E q(x)dm(x)$ is defined, although possibly infinite, for every measurable set E) and if $E = \bigcup_{n=1}^{\infty}\{x: q^n(x) \geqq 0\}$ then $\int_E q(x)dm(x) \geqq 0$. For if $E_j = \{x: q^i(x) < 0,\ 1 \leqq i < j;\ q^j(x) \geqq 0\}$, then for $1 \leqq i < j$ and for any $x \in E_j$, $q^{j-i}(T^ix) = (q^j(x) - q^i(x))/\omega_i(x) \geqq q^j(x)/\omega_i(x) \geqq 0$, so that $T^iE_j \subseteq \bigcup_{k=1}^{j-i}E_k$. For any positive integer n write $F_n = E_n$ and, by induction backward, $F_{n-k} = E_{n-k}(X - \bigcup_{j=n-k+1}^{n}\bigcup_{i=0}^{j-1}T^iF_j)$: it follows that $F_j \subseteq E_j$, $j = 1, \ldots, n$, $\bigcup_{k=1}^{n}E_k = \bigcup_{j=1}^{n}\bigcup_{i=0}^{j-1}T^iF_j$, and the $n(n+1)/2$ sets in the last written double union are pairwise disjoint. Hence

$$\int \bigcup_{k=1}^{n}E_k q(x)dm(x) = \sum_{j=1}^{n}\sum_{i=0}^{j-1}\int_{T^iF_j}q(x)dm(x) = \sum_{j=1}^{n}\sum_{i=0}^{j-1}\int_{F_j}q(T^ix)\omega_{F_j}(x)dm(x) = \sum_{j=1}^{n}\int_{F_j}q^j(x)dm(x) \geqq 0.$$

(*C*) If for any real number c, $E^*(c) = \bigcup_{n=1}^{\infty}\{x: f^n(x) \geqq cg^n(x)\}$ and $E_*(c) = \bigcup_{n=1}^{\infty}\{x: f^n(x) \leqq cg^n(x)\}$ then it follows by applying (*B*) first to

$q(x) = f(x) - cg(x)$ and then to $q(x) = cg(x) - f(x)$, that $\int_{E^*(c)} f dm \geqq c\int_{E^*(c)} g dm$ and $\int_{E_*(c)} f dm \leqq c\int_{E_*(c)} g dm$. It follows also from these inequalities that, although g was not assumed to be integrable, its integrals over $E^*(c)$ and $E_*(c)$ are finite.

(*D*) If $\int_X |f(x)|\, dm(x) = M < \infty$ then for $c > 0$, $\int_{E^*(c)} g dm$ and $\int_{E_*(-c)} g dm$ are both $\leqq M/c$, by (*C*). If F is the set of those points x for which the sequence $\{f^n(x)/g^n(x)\}$ is not bounded then it follows from these inequalities and the relation $F = \bigcap_{c>0} E^*(c) \cup \bigcap_{c>0} E_*(c)$ that $\int_F g(x) dm(x) = 0$. Hence $g(x) = 0$ for almost every x belonging to the invariant set F; Theorem 3 implies that $m(F) = 0$, i.e., that $\{f^n/g^n\}$ is bounded almost everywhere.

(*E*) If $h_*(x)$ and $h^*(x)$ are, respectively, the limit superior and the limit inferior of $f^n(x)/g^n(x)$, then the relation $f^n(Tx)/g^n(Tx) = (f^{n+1}(x) - f(x))/(g^{n+1}(x) - g(x))$ (proved by (*A*)), together with the assumption $\lim_{n\to\infty} g(x) = \infty$ almost everywhere, implies that $h^*(x)$ and $h_*(x)$ are almost everywhere invariant under T.

(*F*) If $M_{rs} = \{x: h_*(x) < r < s < h^*(x)\}$, then $m(M_{rs}) = 0$. For, since (by (*E*)) M_{rs} is invariant under T, the inequalities in (*C*) remain valid if M_{rs} is considered to be the whole space. Since $M_{rs} \subseteqq E_*(r)E^*(s)$, it follows that $s\int_{M_{rs}} g dm \leqq \int_{M_{rs}} f dm \leqq r\int_{M_{rs}} g dm$; since (also by (*C*)) $\int_{M_{rs}} g dm < \infty$, the assumption $r < s$ implies that $\int_{M_{rs}} g dm = 0$. The invariance of M_{rs} and Theorem 3 show that $m(M_{rs}) = 0$.

Since $\{x: h_*(x) < h^*(x)\} = \bigcup_{r,s} M_{rs}$, where the union is extended over all pairs of rational numbers, the conclusion of Theorem 1 follows from (*F*).

The proof shows also that the limit function $h(x)$ is invariant under T (and, of course, measurable). A straightforward adaptation of standard methods can be used to show also that the product $h(x)g(x)$ is integrable, and that for every invariant measurable set E for which $\int_E g dm < \infty$, the identity $\int_E f dm = \int_E h g dm$ holds.

5. If T is non-singular and if there exists a measure m^* invariant under T, such that $m^*(E) = 0$ if and only if $m(E) = 0$, and such that the space X is the union of countably many sets of finite m^* measure, then, at least in the incompressible case, Theorem 1 is an easy consequence of Hopf's theorem. (Whether or not such an m^* always exists is still an open question.[5]) The proof of this statement is similar to, but much simpler than, the proof of Theorem 6.

Under the conditions described, there exists a function $p(x)$, measurable and almost everywhere positive, such that for every measurable set E, $m(E) = \int_E p(x) dm^*(x)$. Since $\int_X f(x) dm(x) = \int_X f(x)p(x) dm^*(x)$, it follows that $f(x)p(x)$ is integrable with respect to m^* and consequently (assuming that T is incompressible) Theorem 5 may be applied to the transformation T, the measure m^*, the integrable function $f^*(x) = f(x)p(x)$,

and the invariantly positive function $g^*(x) = g(x)p(x)$. The conclusion is that $\sum_{i=0}^{n-1} f^*(T^i x)/\sum_{i=0}^{n-1} g^*(T^i x)$ converges to a finite limit almost everywhere with respect to m^* and hence almost everywhere with respect to m. Since, however,

$$\begin{aligned} \int_E \omega_n(x)p(x)dm^*(x) &= \int_E \omega_n(x)dm(x) = m(T^n E) \\ &= \int_{T^n E} p(x)dm^*(x) = \int_E p(T^n x)dm^*(x), \end{aligned}$$

it follows that $p(T^n x) = \omega_n(x)p(x)$ almost everywhere. If the indicated substitution is made in the expressions whose convergence Hopf's theorem asserts, the asserted result follows.[6]

[1] Proof of a recurrence theorem for strongly transitive systems and proof of the ergodic theorem, these PROCEEDINGS, **17**, 650 (1931).

[2] Zu Birkhoff's "Lösung des Ergodenproblems," *Mathematische Annalen*, **107**, 485 (1932).

[3] *Ergodentheorie*, Berlin, 1937, p. 49.

[4] "Ergodic Theorem without Invariant Measure," *Ann. Math.*, **45**, 195 (1944).

[5] This problem will be treated and necessary and sufficient conditions for the existence of such an m^* will be derived in a forthcoming paper, "Invariant Measures," submitted for publication to *Ann. Math.*

[6] I wish to express my thanks to J. C. Oxtoby for his critical reading of the manuscript of this paper and for making several valuable suggestions, and to G. W. Mackey, who called my attention to the fact that by methods similar to those used in this paper it is easy to deduce Theorem 6 from Theorem 7. This shows that the generalization of Hurewicz's theorem in the direction of Hopf's is in reality no generalization at all.